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Leeann Salathe Sticker

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**Nutrient restriction in mares: Plasma metabolite and hormonal
characterization and responses to feeding and metabolic
challenges**

Sticker, Leeann Salathe, Ph.D.

The Louisiana State University and Agricultural and Mechanical Col., 1994

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300 N. Zeeb Rd.
Ann Arbor, MI 48106

**NUTRIENT RESTRICTION IN MARES: PLASMA METABOLITE AND
HORMONAL CHARACTERIZATION AND RESPONSES TO
FEEDING AND METABOLIC CHALLENGES**

A Dissertation

**Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy**

in

The Department of Animal Science

**by
Leeann Salathe Sticker
B.S., Louisiana State University, 1985
M.S., Louisiana State University, 1988
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ABSTRACT

Three experiments were designed to provide a comprehensive reference for the responses of plasma metabolites and hormones to control and restricted intakes of protein and(or) energy and to feed deprivation. The objectives of the first and second experiments were to characterize the effects of protein and(or) energy restriction on glucose, insulin, nonesterified fatty acids (NEFA), urea nitrogen (N), insulin like growth factor-1 (IGF-1), cortisol, triiodothyronine (T_3), thyroxine (T_4), prolactin, and growth hormone (GH) concentrations in plasma on a daily basis, after feeding, and after i.v. administration of glucose or epinephrine. The third experiment was conducted to answer questions of how these plasma constituents, plus β -hydroxybutyrate, lactate, and glucagon, respond to feed deprivation and exercise. In the first experiment, energy restriction increased daily plasma concentrations of NEFA ($P = .0001$) and urea N ($P = .013$), whereas protein restriction decreased ($P = .002$) urea N concentrations. Restriction of protein and(or) energy reduced ($P = .0001$) plasma IGF-1 concentrations. These effects of protein and energy restriction occurred within 24 h of dietary initiation and were consistent (day effect, $P > .1$) throughout the experiment. Plasma NEFA concentrations were altered by energy restriction during feeding (energy x time, $P = .005$) and the epinephrine challenge (energy x time, $P = .06$). Protein restriction increased ($P = .09$) GH episodes during a 14 h feeding period. In the second experiment, immediate responses of a dietary switch were observed for NEFA

(control to restricted; time x diet interaction, $P = .009$; restricted to control; time x diet interaction, $P = .04$). In the third experiment, feed deprivation elevated plasma NEFA, β -hydroxybutyrate, urea N, glucagon, and cortisol (day x diet, $P < .1$), and reduced IGF-1 (day x diet, $P = .04$) concentrations. Feed deprivation increased the plasma glucose (time x diet, $P = .07$) and prolactin (time x diet, $P = .09$), and decreased the NEFA (time x diet, $P = .006$) response to exercise. It was concluded that metabolic and hormonal responses occur within 24 h of dietary changes and that plasma constituents are altered by protein and(or) energy restriction during feeding, glucose, and epinephrine challenges, and also by feed deprivation on a daily basis and during exercise.

CHAPTER I

INTRODUCTION

Nutrition plays a critical role in characterization of plasma metabolites and hormones. Specific nutritional formulations, appropriately quantified in terms of NRC requirements, should be an integral part of every physiologic, endocrinologic, and metabolic study. Therefore, a thorough description of metabolic and hormonal responses to nutrient restriction, deprivation, and maintenance feeding in the adult horse is needed. It is also important to characterize at what point plasma metabolites and hormones respond to altered dietary intakes to appropriately monitor plasma constituents in future studies. The responses of plasma constituents before and after feeding are also critical for determination of appropriate sampling regimens. It is also necessary, for appropriate evaluation of nutritional status, to provide baseline values of various plasma metabolites and hormones during varied nutritional states.

Little information is available in the horse which describes responses of plasma metabolites and hormones to varying nutritional status. Therefore, three experiments were conducted to characterize the responses of plasma glucose, insulin, nonesterified fatty acids (NEFA), urea nitrogen (N), prolactin, insulin like factor-1 (IGF-1), cortisol, triiodothyronine (T_3), thyroxine (T_4), growth hormone (GH), β -hydroxybutyrate, lactate, and glucagon to reduced dietary protein and(or) energy intakes or feed deprivation. The first experiment describes response of

these metabolites and hormones to control or restricted (100 or 50% of NRC requirements) protein and(or) energy intakes, on a daily basis, and relative to feeding and i.v. glucose and epinephrine administration. The second experiment was designed to describe the immediate response (within 24 h) of several plasma metabolites and hormones to protein and energy restriction. The final experiment was then conducted to determine plasma metabolic and hormonal responses to total feed deprivation and relative to exercise.

CHAPTER II

REVIEW OF LITERATURE

Nutrient Restriction

Glucose and Insulin. Varying responses of plasma glucose and insulin concentrations are observed to protein and(or) energy restriction. Dietary protein and energy restriction did not influence plasma glucose or insulin concentrations prior to feeding in weanling Thoroughbreds fed 80% of their protein and energy requirements (Glade et al., 1984). Prunier et al. (1993) also reported no variations in plasma glucose and insulin concentrations in peripubertal gilts fed near or 50% of ad libitum intake. In contrast to studies with nonruminants, plasma glucose and insulin concentrations were lower prior to feeding in steers restricted to intakes approximately half of the levels fed to control steers (Blum et al., 1985). Ellenberger et al. (1989) also observed reduced glucose concentrations in growing steers fed to gain only .37 kg/d as compared to steers fed to gain 1.4 kg/d. In addition, plasma insulin concentration has been positively correlated to protein and energy, or DM intake, in young sheep (Bassett et al., 1971; Waghorn et al., 1987) and was positively correlated to protein intake in young bulls fed isocaloric diets (Martin et al., 1979). Waghorn et al. (1987) suggested that protein was the principal determinant of plasma insulin concentrations. Other research reported no alterations of plasma insulin concentrations in lactating dairy cows fed isocaloric diets at either 80 or 100% of CP requirements (Chew et al., 1984a). Also, similar

glucose concentrations were observed in adult cows fed isonitrogenous, restricted energy diets (Schrack et al., 1990).

Glucagon. Plasma glucagon concentrations would be expected to increase with nutrient restriction to increase mobilization of glucose via glycogenolysis and gluconeogenesis (Guyton, 1991). Reductions in dietary protein did not alter plasma glucagon concentrations in goats (Gaskins et al., 1991; Sahlu et al., 1992).

Nonesterified Fatty Acids. Plasma NEFA concentrations were reported for horses and ponies prior to feeding (Robie et al., 1975; Luther et al., 1981) and for exercising horses fed either corn or alfalfa (Zimmerman et al., 1992). Reduction of energy intake elevates plasma NEFA concentrations as a result of increased lipolytic activity. This relationship can be directly drawn because there is a linear relationship between plasma NEFA concentrations, NEFA turnover, and NEFA metabolism (Issekutz et al., 1965). Energy intakes were inversely related to plasma NEFA concentrations in cattle (Holmes and Lambourne, 1970). Energy restriction to 78% of NRC requirements for lactating cows increased plasma NEFA concentrations (McGuire et al., 1992a), whereas reducing energy intake from 26.5 to 15.2 Mcal/d in lactating cows did not influence plasma NEFA concentrations (Schrack et al., 1990). Reduction of dietary intake also increased plasma NEFA concentrations in gilts (Prunier et al., 1993) and steers (Blum et al. 1985). However, altering growth rate of steers from 1.4 to .37 kg/d did not affect plasma NEFA concentrations (Ellenberger et al., 1989). In humans on restricted energy intake, the combination of reduced plasma insulin concentrations and increased GH

and epinephrine concentrations result in fatty acid mobilization from adipose tissue (Guyton, 1991).

Urea Nitrogen. Plasma urea N concentrations appears to be influenced by many dietary factors including protein intake, energy intake, and level of feeding (Hammond, 1983). Increased protein intake results in protein wastage, whereas reduced energy intake permits elevated use of protein for energy; both situations elevate plasma urea N concentrations. Indeed, plasma urea N concentration was increased as dietary protein increased in horses (Fonnesbeck and Symons, 1969; Patterson et al., 1985) and ruminants (Preston et al., 1965; Hammond, 1983). Likewise, low protein intakes also reduced plasma urea N concentrations in lambs (Preston et al., 1965) and pigs (Eggum, 1970). Increased energy intake, with isonitrogenous diets, decreased plasma urea N concentrations in ruminants (Hammond, 1983). The ratio between protein and energy intake might be more important than either protein or energy intake alone (Hammond, 1983). In contrast, Ellenberger et al. (1989) and Blum et al. (1985) reported no variation of plasma urea N concentrations in growing steers fed reduced feed intakes.

Prolactin. Researchers have reported varying result of the influences of feed restriction on plasma prolactin concentrations in several species. Plasma prolactin concentrations were not altered in weanling lambs fed either to grow normally or maintain body weight (Foster et al., 1989). Forbes et al. (1975) reported higher prolactin concentrations during short day lengths and no differences during long day lengths in growing lambs fed ad libitum as compared with restricted diets.

Cosgrove et al. (1991) reported elevated plasma prolactin concentrations with feed restriction (30% of ad libitum intake) as compared with ad libitum feeding in prepubertal gilts. On the other hand, during periods without feed, plasma prolactin concentrations have been shown to be low in goats (Bryant et al., 1970), cattle (McAtee and Trenkle, 1971c), humans (Quigley et al., 1981), and horses (DePew et al., 1994). In sheep and cattle, exogenous prolactin has been shown to increase growth or nitrogen retention (Bauman et al., 1982). Prolactin may have similar somatotrophic effects by promoting muscle protein accretion and(or) increasing lipolysis in the adipose tissue. It is unclear what influences feed restriction has on plasma prolactin concentrations and prolactin's metabolic role during altered nutritional status.

Insulin Like Growth Factor-1. Plasma IGF-1 concentrations have been reported in foals at birth and 4 mo of age to average 219 and 210 ng/mL, respectively (Tremblay et al., 1993). McGuire et al. (1992b) summarized reports for humans, rats, and ruminants, which show that plasma IGF-1 concentrations decrease with severe nutritional deficiencies and feed withdrawal, and that plasma GH concentrations do not stimulate the release of IGF-1 under these conditions. Elsasser et al. (1989) reported depressed plasma IGF-1 concentrations with nutritional restriction (holding weight gain to a minimum) in steers in addition to the uncoupling of the stimulatory effect of GH on IGF-1. Reduced plasma IGF-1 concentrations with nutritional deficiencies (resulting in weight loss) were also reported in heifers (Houseknecht et al., 1988; Granger et al., 1989).

It is not clear if the reduction in plasma IGF-1 concentrations due to nutritional deficiencies is a result of specifically protein or energy deficiency, or a combination. Restriction of protein or energy alone or in combination at 80% of requirements for lactation cows did not alter plasma IGF-1 concentrations (McGuire et al., 1992a). In rats, protein restriction appears to be related to decreased somatomedin activity and plasma concentrations (Reeves et al., 1979; Prewitt et al., 1982). Kriel et al. (1992) suggested that protein intake could directly influence plasma IGF-1 concentrations by altering the supply of amino acids to the liver. Cree and Schalch (1985) reported reduced plasma IGF-1 concentrations in rats fed an isocaloric and isonitrogenous diet with one third as much lysine as a control diet. However, in young chickens, Rosebrough et al. (1989) observed decreased plasma IGF-1 concentrations with decreased energy intake, with either constant or varied protein levels.

Possible reasons for decreased plasma IGF-1 concentrations with nutritional deficiencies were related to the binding protein (McGuire et al., 1992b), affinity or GH receptor numbers at the liver (Baxter et al, 1981; Breier et al., 1988) or transcription or translation (Thissen et al., 1991). Breier et al. (1988) reported that the presence of high-affinity somatotrophic binding sites is determined by nutritional status.

Cortisol. Plasma cortisol concentrations previously reported for horses average 33 to 70 ng/mL (James et al., 1970; Glade et al., 1984; Stull and Rodiek, 1988; Sojka et al., 1993). Glade et al. (1984) reported higher plasma cortisol

concentrations in weanlings fed 160 vs 80% of their protein and energy requirements (60.8 vs 48.7 ng/mL, respectively). In cattle, acute exposure to elevated temperatures for several hours increased, whereas prolonged exposure (24 d) decreased plasma corticosteroid concentrations (Alvarez and Johnson, 1973). Because of the metabolic role of cortisol (increasing gluconeogenesis, lipolysis, and protein catabolism; Guyton, 1991), it would seem that nutrient restriction would increase circulating cortisol concentrations to facilitate nutrient mobilization; however, varying results have been observed. Indeed, some researchers observed increased plasma cortisol concentrations during hypoglycemia in horses (James et al., 1970) and 5 h after feeding peripubertal gilts a diet which was 50% ad libitum intake (Prunier et al., 1993). In contrast, Schrick et al. (1990) reported similar daily peripheral blood cortisol concentration in cows (100 d postcalving) fed either at high or low energy intakes. And still others have reported higher plasma cortisol concentrations in lambs fed high as compared to moderate protein levels at two environmental temperatures (Ghani, 1988).

Thyroid Hormones. Thyroid function has been reported to decrease with nutrient restriction, although varying results are observed. In horses, Glade and Reimers (1985) reported no differences in plasma T_3 or T_4 concentrations in weanling foals fed either 70, 100 or 130% of their protein and energy requirements. Glade et al. (1984) also observed similar plasma T_4 concentrations before feeding in weanlings fed either 80 or 160% of their protein and energy requirement. Reduced plasma T_3 and T_4 concentrations have been reported with reduced feed

intake in steers (Blum et al., 1985) and rats (Schalch and Cree, 1985). Likewise, Blum et al. (1980) observed reduced plasma T_3 concentrations in adult sheep fed at reduced levels, but no change in plasma T_4 concentrations. Ellenberger et al. (1989) observed no change in T_3 , but lowered T_4 in growing steers fed to gain only .37 kg/d as compared to steers fed to gain 1.4 kg/d. Thyroid function appears to have significant influences on GH action, and the uncoupling of GH and IGF-1 with nutritional deficiencies (Elsasser et al., 1989) appears to be associated with reduced plasma T_3 concentrations (Cabello and Wrutniak, 1989).

Growth Hormone. Plasma GH concentrations are pulsatile or episodic in nature in horses and similar to patterns for other species (Thompson et al. 1992; Stewart et al., 1993; DePew et al., 1994). Elevated circulating GH concentrations have been associated with dietary protein and(or) energy restriction in other species for the conservation of protein and carbohydrates at the expense of fat stores (Guyton, 1991). Protein restriction elevated plasma GH concentrations in yearling rams (Barenton et al., 1987), while energy restriction increased GH concentrations in growing broiler chickens (Rosebrough et al., 1989). This GH response is not evident in rats (Achilles et al., 1982; Schalch and Cree, 1985) or with mild restriction in cattle (Chew et al., 1984a; Elsasser et al., 1989). Both protein and energy restriction increased GH concentrations in lactating dairy cows (deBoer et al., 1985), growing beef heifers (Granger et al., 1989), infants (Muzzo and Alcazar, 1985), and adult ewes (Thomas et al., 1990).

Responses to Feeding

Glucose and Insulin. In horses, plasma glucose and insulin concentrations increased within 1 to 2 h and returned to prefeeding levels within 4 to 6 h after the beginning of a meal (Glade et al., 1984; Stull and Rodiek, 1988; and DePew et al., 1994). Glade et al. (1984) reported that plasma glucose and insulin concentrations remained elevated longer following a meal (fed twice per day) in which young horses were fed to meet 160% as compared with 80% of their daily protein and energy requirements. A meal consisting of alfalfa/corn or corn to meet 25% of the daily DE requirements for adult horses elevated plasma glucose and insulin concentrations to a greater extent than an isocaloric meal of alfalfa or corn/corn oil (Stull and Rodiek, 1988). In peripubertal gilts, rises in plasma glucose and insulin concentrations were similar after feeding regardless of intake (Prunier et al., 1993). Buonomo and Baile (1991) observed no response of plasma glucose concentrations in 70 kg barrows, but did observe increased plasma insulin concentrations with feeding. Ruminants, on the other hand, exhibit a slow rise in plasma glucose concentrations after feeding, peaking at 4 to 8 h (McAtee and Trenkle, 1971a,b; McCarthy et al., 1992). Hepatic gluconeogenesis, following absorption of propionate, lactate, and amino acids (Herdt, 1988), provides this delayed elevation in ruminants, whereas in horses, direct glucose absorption increases plasma concentrations within 1 h following feeding. Rapid increases of plasma insulin concentrations to feeding were observed in calves (McAtee and Trenkle, 1971b) and in lambs and adult sheep (Bassett, 1974b).

Glucagon. Plasma glucagon concentrations decrease during hyperglycemia and increase during hypoglycemia (Brockman, 1977). However, increased circulating amino acids, as observed after feeding in the horse (Johnson and Hart, 1974; DePew et al., 1994), stimulate glucagon secretion (Kuhara et al., 1991) for the rapid conversion of amino acids to glucose (Guyton, 1991). A stimulatory effect of feeding on plasma glucagon concentrations was observed in sheep (Bassett, 1972), goats (de Jong, 1981b), cows in the early lactation period (deBoer et al., 1985), and horses (DePew et al., 1994). However, plasma glucagon concentrations did not change in relation to feeding in goats (de Jong, 1981a) and in cows during the dry period, in a feed restriction induced ketonemia, or during a recovery period (deBoer et al., 1985).

Nonesterified Fatty Acids. Feeding influences plasma NEFA concentrations, but this is dependent on energy intake. As discussed previously, energy restriction or decreased intake elevates plasma NEFA concentration; however, researchers have observed varying results. These varying results might be attributed to the sampling time relative to feeding or number of feedings. DePew et al. (1994) reported a large reduction in plasma NEFA concentrations after feeding in horses that were fed once daily. Prunier et al. (1993) observed varying responses of NEFA concentrations to feeding in peripubertal gilts fed twice daily at levels close to ad libitum or restricted to half ad libitum intake. In both groups, plasma NEFA concentrations were reduced after feeding (16 h after previous meal) at 160 d of age, while at 210 d of age, when control gilts were potentially consuming intakes

more closely matching their requirements, only restricted gilts had lower plasma NEFA concentrations following feeding. In lactating cows, plasma NEFA concentrations were highest immediately before and lowest 4 to 6 h after a meal (deBoer et al., 1985) regardless of intake (ad libitum vs 50% ad libitum).

Urea Nitrogen. In ruminants, small but significant effects of feeding on plasma urea N concentrations are frequently observed (Coggins and Field, 1976; Manston et al., 1981; Hammond, 1983; McCarthy et al., 1992). However, in pigs, a more marked increase in plasma urea N concentrations is observed in response to feeding (Eggum, 1970). Horses seem to more closely resemble ruminants than nonruminants with minor but significant increases in plasma urea N concentrations associated with feeding. This phenomenon was reported by Depew et al. (1994). In contrast, when sampling every 90 min, Hintz and Schryver (1972) found no change in plasma urea N concentrations in ponies after feeding.

Prolactin. Plasma prolactin concentrations have been shown to increase as a result of feeding and have been linked to amino acids and protein (Carlson, 1989), opiate peptides (Zioudrou et al., 1979), and neurotransmitter precursors in the meal (Ishizuka et al., 1983). DePew et al. (1994) reported elevated plasma prolactin concentrations from 30 min to 4 h after feeding. Bryant et al. (1970) reported increased plasma prolactin concentrations in goats within 30 min after feeding, whereas McAttee and Trenkle (1971c) observed in heifers that feeding caused a steady rise in plasma prolactin concentrations to a maximum 6 h after meal initiation. In the woman, a meal at lunch and supper was reported to increase

plasma prolactin concentrations within 45 min (Quigley et al., 1981). Others have reported increased plasma prolactin concentrations after feeding in lambs (Godden and Weekes, 1981) and rats (Bronson and Heideman, 1990).

Insulin Like Growth Factor-1. Responses of plasma IGF-1 concentrations to feeding in horses has not been reported and little information is available in other species. Buonomo and Baile (1991) reported elevated plasma IGF-1 concentrations within 4 h after feeding in 70 kg barrows which were previously held without feed for 48 h.

Thyroid Hormones. Because of the role of thyroid hormones in increasing gastrointestinal tract secretion and motility and in carbohydrate (increased glucose absorption and tissue uptake) and fat metabolism (Guyton, 1991), increases in plasma thyroid hormone concentrations following feeding would appear consistent with their actions. Indeed, Youket et al. (1985) and Glade et al. (1984) reported increased plasma T_3 concentrations in adult ponies fed once daily and increased plasma T_4 concentrations in weanling horses fed twice daily, respectively, in response to a meal. Glade and Reimers (1985) reported variations in plasma T_3 and T_4 responses to feeding that depended on age and on protein and energy intake. In 70 kg barrows previously held without feed for 48 h, increases in plasma T_3 and T_4 concentrations were observed within 2 h after feeding (Buonomo and Baile, 1991).

Growth Hormone. If feeding stimulates GH secretion, perhaps the resulting elevated plasma GH concentrations might serve to increase amino acid transport,

glycogen deposition, or synthesis of proteins (Guyton, 1991). On the other hand, if feeding decreases GH secretion, then related reasons might include reduction in lipolysis or removal of its negative influence related to glucose usage as an energy fuel (Guyton, 1991). However, DePew et al. (1994) reported no relationship between feeding and plasma GH concentrations in mares and stallions. In other species, varying responses of plasma GH concentrations in relation to feeding have been reported. Plasma GH concentrations increased after feeding in growing lambs (Symonds et al., 1989) and in adult dairy cows (de Boer et al., 1985) and decreased after feeding in adult sheep (Basset, 1974a; Trenkle, 1989) and goats (Tindal et al., 1982).

Intravenous Glucose Tolerance Test (IVGTT)

Glucose and Insulin. Glucose metabolism in horses has been shown to be more comparable to ruminants when horses are fed high forage diets, but more comparable to the nonruminant pattern when they are fed high grain diets (Argenzio and Hintz, 1972). Garcia and Beech (1986) observed a lower insulin and glucose response to glucose administration in adult horses fed a mixture of timothy and alfalfa hay ad libitum as compared to those fed the hay plus a grain supplement. Cole et al. (1993) noted that glucose fractional removal rates were faster and the insulin response curve area was not different after a glucose load in fed as compared with unfed (3 d) lambs. In yearling sheep, a more rapid decline

in plasma glucose and insulin concentrations after glucose infusion was observed during higher feed intakes (Waghorn et al., 1987).

Prolactin. A response of plasma prolactin concentrations to glucose administration might indicate that the feed induced rise in glucose or the associated insulin elevation is related to elevated prolactin following feeding. However, glucose metabolism appears to be unrelated to the feed induced prolactin response as McAtee and Trenkle (1971c) and Bryant et al. (1970) reported that glucose injection had no influence on plasma prolactin concentrations in 5 mo old heifer calves and goats, respectively. On the other hand, Cole et al. (1993) observed that lambs which were fed to meet their requirements had reduced plasma prolactin concentrations following glucose administration, whereas lambs held without feed for 3 d had increased plasma prolactin concentrations following glucose administration.

Epinephrine Challenge

Glucose and Nonesterified Fatty Acids. Catecholamines are important during reduced feed intake for the mobilization of nutrients via increased glycogenolysis and fatty acid mobilization; however, their role is diminished during feed deprivation for the conservation of body tissues (Landsberg and Young, 1978). In support of this, Frohli and Blum (1988) reported decreased circulating norepinephrine and no change in epinephrine concentrations during feed deprivation in steers. In contrast, Beer et al. (1989) reported elevated epinephrine

and norepinephrine concentrations during 72 h without food in humans. During 3 to 4 d of feed deprivation, Blum et al. (1982) and Frohli and Blum (1988) reported increased plasma NEFA and decreased glucose responses to epinephrine infusions in cattle.

Growth Hormone. Varying responses of plasma GH concentrations to epinephrine administration have been reported. In adult stallions, epinephrine increased plasma GH concentrations (Thompson et al., 1992) and in yearling wether sheep epinephrine decreased plasma GH concentrations (Bassett et al., 1971).

Feed Deprivation

Glucose and Insulin. Baetz and Pearson (1972) and Freestone et al. (1991) reported no changes in plasma glucose concentrations during 9 or 5 d of feed deprivation in adult ponies. Similarly, Rose and Sampson (1982) and Freestone et al. (1991) observed similar plasma insulin concentrations during a 4 or 5 d period without feed in horses and ponies. Likewise, in swine, little variation of plasma glucose concentrations were observed during 48 h of feed deprivation (Baetz and Mengeling, 1971). Absolute requirements of glucose for the nervous system, erythrocytes, and brain result in a precise regulatory mechanism for the conservation of blood glucose (Murry et al., 1993). Glucose is the major stimulator of insulin secretion; however, amino acids have also been implicated as insulin secretagogues (Davis, 1972; Chew et al., 1984b; Vicini et al., 1988). In man, plasma

glucose (Ho et al., 1988) and plasma glucose and insulin concentrations (Beer et al., 1989) have decreased during periods without food intake. Reports in ruminants have shown decreased plasma glucose (Blum et al., 1981; Rule et al., 1985; Heitmann et al., 1986) and insulin (Bassett, 1974b) concentrations during feed deprivation as compared to the fed state.

Glucagon. Plasma glucagon concentrations have been reported to increase during 3 d of feed deprivation in humans (Beer et al., 1989) and to remain unchanged during 3 or 8 d without feed in ruminants (Rule et al., 1985; Heitmann et al., 1986). An elevation of plasma glucagon concentrations with feed deprivation would likely ensue from a reduction in postprandial plasma glucose concentrations and(or) sympathetic stimulation. Bassett (1971) reported elevated plasma glucagon concentrations after epinephrine administration in sheep, and Freeman (1980) concluded that glucagon is a stress hormone in chickens.

Nonesterified Fatty Acids. In horses, plasma NEFA concentrations increase within 2 to 3 d after feed removal (Baetz and Pearson, 1972; Naylor et al., 1980; Rose and Sampson, 1982) resulting from increased lipolysis and(or) decreased lipogenesis, related to changes in either circulating insulin, glucagon, epinephrine, norepinephrine (Rose and Sampson, 1982), GH, thyroid stimulating hormone, or adrenocorticotrophic hormone (ACTH; Guyton, 1991). Similar responses of plasma NEFA concentrations to feed deprivation have been reported for swine (Baetz and Mengeling, 1971), cattle (Rule et al., 1985; Ward et al., 1992), sheep (Heitmann et al., 1986), and humans (Ho et al., 1988).

β -Hydroxybutyrate. Increases in plasma β -hydroxybutyrate concentrations resulting from increased hepatic production (Heitmann et al., 1986) has been reported in adult geldings and mares after 72 h of feed deprivation (Rose and Sampson, 1982). Similar results were also observed in steers (Blum et al., 1981; Rule et al., 1985), adult sheep (Heitmann et al., 1986), and man (Ho et al., 1988; Beer et al., 1989).

Lactate. In the horse, plasma lactate concentrations have been reported to continually increase during 9 or 4 d without feed (Baetz and Pearson, 1972; Rose and Sampson, 1982). Increased circulating lactate concentrations would likely result from increased lactate production via muscle glycolysis for support of hepatic gluconeogenesis (Murry et al., 1993).

Urea Nitrogen. In the horse, Baetz and Pearson (1972) reported reduced plasma urea N concentrations during 3 d, whereas Patterson et al. (1985) reported increased plasma urea N concentrations during 4 d of feed deprivation. Others have reported increases in plasma urea N concentrations during 115 h of feed deprivation in swine (Baetz and Mengeling, 1971) and during 5 or 7 d of feed deprivation in cattle (Blum et al, 1981; Blum and Kunz, 1981). Rule et al. (1985) observed an elevation after 2 d then a reduction in plasma urea N concentrations during the remaining 6 d of feed deprivation in cattle. Increased plasma urea N concentrations are probably the result of increased mobilization of protein to serve as an energy fuel.

Prolactin. Plasma prolactin concentrations appear to be low during feed deprivation. DePew et al. (1994) reported low plasma prolactin concentrations after 15 h without feed. Others have reported similar results in goats held without feed for 8 h (Bryant et al., 1970), heifer calves for 60 h (McAttee and Trenkle, 1971c), and rats for 72 h (Takahashi et al., 1986).

Insulin Like Growth Factor-1. Researchers have observed reductions in plasma IGF-1 concentrations during feed deprivation in many species. Buonomo and Baile (1991) reported a 53% decrease in plasma IGF-1 concentrations in swine 48 h after feed removal and increased plasma IGF-1 concentrations with refeeding. Likewise, plasma IGF-1 concentrations decreased with feed withdrawal in rats (Phillips and Young, 1976), humans (Merimee et al., 1982), and bulls and steers (Ward et al., 1992). This reduction supports the uncoupling of the stimulatory effect of GH on IGF-1 concentrations as is similarly seen during reduction of intake (McGuire et al., 1992b).

Cortisol. Plasma cortisol concentrations would be expected to rise during feed deprivation related to stress and for its use in nutrient mobilization; however, varying responses are observed. In adult ponies, plasma cortisol concentrations did not vary during a 5 d (Freestone et al., 1991) or during a 4 d period without feed (Rose and Sampson, 1982). On the other hand, 72 h of food deprivation in humans increased plasma cortisol and ACTH concentrations (Beer et al., 1989).

Thyroid Hormones. Reduction of thyroid hormones during feed deprivation was observed in several species and is attributed to either a reduction in thyroid

stimulating hormone, or to decreased thyroid hormone secretion via elevated circulating corticosteroid concentrations (Beer et al., 1989). Feed deprivation reduced both plasma T_3 and T_4 concentrations in sheep (Blum et al., 1980), cattle (Blum and Kunz, 1981; Blum et al., 1981; Tveit and Larsen, 1983), and pigs (Buonomo and Baile, 1991). Beer et al. (1989) reported elevated plasma T_4 and thyroid stimulating hormone concentrations, but no change in plasma T_4 concentrations in humans during withdrawal of food.

Growth Hormone. Feed deprivation generally elevates plasma GH concentrations, although varying responses are observed. Elevated plasma GH concentrations during feed deprivation were observed in lambs and adult sheep (Bassett, 1974b; Cole et al., 1988), 70 kg barrows (Buonomo and Baile, 1991), and adult humans (Ho et al., 1988; Beer et al., 1989). In feed-deprived steers, Rule et al. (1985) reported reduced plasma GH concentrations, whereas in calves, McAtee and Trenkle (1971a) reported no alterations of plasma GH concentrations.

Response to Exercise

Nonesterified Fatty Acids. In the horse, plasma NEFA concentrations increase during exercise or recovery (Snow and Mackenzie, 1977; Deldar et al., 1982; Rose and Sampson, 1982; Miller-Graber et al., 1991; Lawrence et al., 1993). Exercise-induced elevation in plasma NEFA concentration for the use as metabolic fuel results from increased lipolysis, via an insulin-related reduction in lipogenesis. Miller-Graber et al. (1991) observed that plasma NEFA concentrations were not

influenced before, during, or after exercise in relation to varying protein intakes. However, during a 12 to 15 h period of feed deprivation, elevated plasma NEFA concentrations were decreased during a warm up, increased during a walking phase then decreased again during a high intensity phase of exercise (Lawrence et al., 1993). Plasma NEFA concentrations are also influenced during exercise in relation to the training status; Issekutz et al. (1965) reported no exercise-related change in plasma NEFA concentrations in untrained dogs, but observed increased plasma NEFA concentrations and turnover in trained dogs.

Lactate. Exercise may elevate circulating lactate concentrations because of increased anaerobic metabolism via muscle or liver glycogenolysis, or glycolysis, and(or) decreased removal (Astrand and Rodahl, 1986). Deldar et al. (1982) and Rose and Sampson (1982) reported elevated plasma lactate concentrations with exercise in horses. This elevated lactate response was not influenced by a 12 to 15 h period of feed deprivation or ingestion of corn 3 h before exercise (Lawrence et al., 1993). Nevertheless, elevated protein intakes reduced plasma lactate concentrations 5 min after a 15 min exercise bout (Miller-Graber et al., 1991). Still, the state of training must be taken into consideration; a greater response of plasma lactate concentrations during exercise has been observed in untrained compared to trained dogs (Issekutz et al., 1965) and horses (Snow and Mackenzie, 1977).

Glucose. Variations in the plasma glucose response to exercise has been observed in the horse. Lawrence et al. (1993) reported a reduction in circulating glucose concentrations after exercise in horses which consumed various amounts of

corn 2.5 to 3 h before the exercise bout; however, plasma glucose concentrations remained relative stable after exercise when horses were previously held without feed for 12 to 15 h. Others have reported no fluctuations in plasma glucose concentration after exercise in the horse (Snow and Rose, 1981; Deldar et al., 1982; Rose and Sampson, 1982), whereas Snow and Mackenzie (1977) observed increased plasma glucose concentrations during endurance exercise in horses. The inconsistent responses are likely related to either time of sampling relative to initiation of the exercise or to the previous feeding. Elevation of plasma glucose concentrations has been observed through 8 h after feeding in horses fed once per day (DePew et al., 1994). Besides the dietary influences, circulating glucose concentrations also depend on the balance between glucose uptake by the working muscle and the rate of hepatic glycogenolysis and gluconeogenesis (Snow and Mackenzie, 1977).

Growth Hormone and Prolactin. Exercise-related increases in plasma GH and prolactin concentrations have been reported in horses (Colborn et al, 1991b; Thompson et al., 1992) and man (Luger et al., 1992). Saini et al. (1990) and Kraemer et al. (1993) reported elevated plasma GH concentrations, but no responses in plasma prolactin concentrations during exercise in man. Both Luger et al. (1992) and Kraemer et al. (1993) observed no variations in the response of plasma GH and prolactin concentrations to exercise with state of training in man. However, there appears to be a intensity dependent stimulation of GH and prolactin (Luger et al., 1992), with this in part related to increased circulating

lactate concentrations. Other mechanisms postulated to explain the plasma GH and prolactin response to exercise included brain neurotransmitters (Chiodini and Liuzzi, 1979) and sympathetic stimulation (Thompson et al., 1992).

For assessing pituitary GH reserves, it appears that exercise is a more potent stimulus for GH secretion than is insulin hypoglycemia, arginine infusion, or L-DOPA administration, and may be as predictive as insulin hypoglycemia (Sutton and Lazarus, 1976). Thompson et al. (1992) also reported a greater plasma GH concentration response to exercise in stallions than either sexual stimulation, restraint via a twitch, or epinephrine administration.

Cortisol. Exercise is associated with the activation of the hypothalamo-pituitary-adrenocortical system because of the related stress (Malinowski et al., 1993). Exercise increased plasma cortisol concentrations in horses during or following exercise (Martinez et al., 1988; Colborn et al., 1991b; Malinowski et al., 1993).

Increased plasma GH (Thompson et al., 1992), epinephrine, norepinephrine (Snow and Rose, 1981), glucagon, cortisol, and lactate concentrations and decreased plasma insulin concentrations during exercise (Lucke and Hall, 1980) collectively initiate nutrient mobilization through gluconeogenesis and lipolysis. Elevated plasma prolactin concentrations might function, in part, for the conservation of tissue protein; increases in growth or nitrogen retention in sheep and cattle have been observed with exogenous prolactin (Bauman et al., 1982).

Rationale of Present Research

The present research was designed to answer questions related to how variations in nutrient intake influence plasma metabolites and hormones and if these varying intakes prompt diverse responses during feeding and metabolic challenges used in the horse. The basic rationale of these experiments began with questions related to how plasma GH responds to reduced protein and(or) energy intakes as well as during feed deprivation in the horse. These questions led to further questions related to the responses of other plasma metabolites and hormones under these situations. A review of the literature in this area in horses led to the conclusion that a comprehensive series of references which answered these questions was essential in the horse.

The first experiment (Chapters III and IV) was designed to answer the following questions: 1) Do plasma concentrations of glucose, insulin, NEFA, urea N, prolactin, IGF-1, cortisol, thyroid hormones, and GH vary with two levels of protein and(or) energy intake (100 and 50% of maintenance requirements); 2) Do these varying intakes of protein and(or) energy influence the responses of plasma glucose, insulin, and prolactin to an IVGTT and of plasma glucose, NEFA, and GH to an epinephrine challenge; and 3) Can these plasma constituents be used to monitor the nutritional status of horses either in a clinical setting or for use by nutritionists? Because plasma NEFA, urea N, and IGF-1 concentrations were altered within the first 24 h after dietary initiation, the second experiment (Chapter V) was designed to characterize more fully these immediate changes to dietary

protein and energy reduction (pretreatment samples were omitted in the first experiment). The second experiment also was designed to answer the question of whether these immediate changes would be observed with a return to control protein and energy intakes. Plasma glucagon was also measured in the second experiment because of the limited glucagon data available in the horse. The final experiment (Chapter VI) was then conducted to answer questions related to how these plasma constituents, including β -hydroxybutyrate, lactate, and glucagon, respond to feed deprivation. Exercise was used to estimate pituitary stores of GH and also to answer questions of how feed deprivation influences plasma NEFA, lactate, glucose, prolactin, and cortisol responses to exercise in the horse.

CHAPTER III

DIETARY PROTEIN AND(OR) ENERGY RESTRICTION IN MARES:
PLASMA GLUCOSE, INSULIN, NONESTERIFIED FATTY ACID,
AND UREA NITROGEN RESPONSES TO FEEDING,
GLUCOSE, AND EPINEPHRINE

Introduction

A thorough description of the hormonal and metabolic responses to alterations in dietary protein and(or) energy levels in adult horses is needed for our understanding of the metabolic significance of diet formulation, as well as the impact of nutrition in physiologic studies. Glade et al. (1984) studied plasma concentrations of glucose, insulin, cortisol, and T_4 in weanling Thoroughbreds fed at 80 or 100% of protein and energy requirements. Others reported metabolic and hormonal responses to a complete pelleted meal or common equine dietary ingredients (Ralston and Baile, 1982; Stull and Rodiek, 1988; DePew et al. 1994). Jacobs and Bolton (1982) and Garcia and Beech (1986) observed that the glucose and insulin response to a glucose tolerance test was altered with hay versus grain-based diets. Epinephrine administration has been used in horses as a growth hormone secretagogue (Thompson et al., 1992) and to evaluate the composition of sweat during exercise (Kerr and Snow, 1983). The objective of the present experiment was to characterize the effects of protein and(or) energy restriction on

glucose, insulin, NEFA, and urea N concentrations in plasma on a daily basis, after feeding, and after i.v. administration of glucose or epinephrine.

Experimental Procedures

Animals and Dietary Treatments. Sixteen light horse mares (8 to 9 yr of age; 457 to 579 kg initial BW), grazing native grass pasture, were stratified by weight (4 horses/treatment) to one of four dietary treatments in a completely randomized design with a 2 x 2 factorial arrangement of treatments. Dietary treatments were formulated with the aim of meeting either 100 (control) or 50% (restricted) of the CP and DE requirement for maintenance (Table 3.1; NRC, 1989); actual intakes averaged 98 and 55% (Table 3.2). The experiment was conducted during the months of November and December, 1992. Horses were offered Alicia bermudagrass hay at .6 kg of DM/100 kg of BW, which provided 30% of the CP requirement in the control diet. The bermudagrass hay (DM basis) contained 6.5% CP, 45.2% ADF, 65.0% NDF, and was calculated to contain 1.78 Mcal DE/kg (NRC, 1982). Supplements were formulated and added in varying amounts (.6 and .22 kg of DM/100 kg of BW for control and restricted energy, respectively) to provide the appropriate amount of CP and DE (Tables 3.1 and 3.2). Supplements were formulated to provide similar amounts of cottonseed hulls and to at least meet the requirements for Ca, P, Mg, Na, K, and S. Before initiation of the experiment, horses were adapted in the pasture to the hay for 10 d and to the control supplement for 5 d. During the experiment, horses were housed in covered

Table 3.1. Ingredient composition and chemical analysis of dietary supplements. Adult mares were fed 100 (control) or 50% (restricted) of protein and(or) energy requirement

Item	Control Protein		Restricted Protein	
	Control Energy	Restricted Energy	Control Energy	Restricted Energy
Ingredient composition, % of DM				
Chopped corn	57.9	1.0	38.1	46.1
Cottonseed hulls	15.0	25.0	15.0	25.0
Corn gluten meal	11.8	56.7		11.1
Molasses	15.0	15.0	15.0	15.0
Cornstarch			31.2	
Oystershell flour	.3			
Dicalcium phosphate		2.1	.7	2.7
Magnesium oxide		.2		.1
Chemical analysis, DM basis				
CP, %	15.5	40.5	5.7	13.7
DE, Mcal/kg	3.5	3.3	3.5	3.2

Table 3.2. Daily intake and BW change. Adult mares were fed 100 (control) or 50% (restricted) of protein and(or) energy requirement

Item	Control protein		Restricted protein		SE
	Control Energy	Restricted Energy	Control Energy	Restricted Energy	
n	4	3	4	4	
Initial BW, kg	510	515	502	511	
Intake					
DM, kg/d					
Hay	3.06	3.09	3.01	3.06	
Supplement	3.04	1.16	2.98	1.14	
CP, g/d					
Hay	200	202	197	201	
Supplement	474	453	165	160	
DE, Mcal/d					
Hay	5.44	5.50	5.36	5.46	
Supplement	10.70	3.74	10.53	3.60	
BW change, kg/d					
Day					
9-18 (9 d) ^a	.22	-.41	.17	-.33	.27
18-25 (7 d)	-.18	-.52	-.54	-.36	.24
25-33 (8 d)	-.16	-.54	-.19	-.28	.29
All days (9-33; 24 d) ^b	-.02	-.49	-.16	-.32	.14

^aEnergy effect (P=.07).

^bEnergy effect (P=.05).

stalls where they were offered their ration of hay and supplement in two equal portions at 0800 and 1600 and had continual access to water.

Weighing and Blood Sampling. Mares were weighed at 0800 (16 h after the afternoon feeding) on d 1, 9, 18, 25, and 33 of the experiment (at 0800 on d 1 mares received their first treatment meal). Metabolic status was monitored by measurement of plasma glucose, insulin, NEFA, and urea N daily for 5 d beginning on d 2, and then every third day after d 6. Blood (7 mL) was drawn at 0800 (16 h after feeding) by jugular venipuncture into evacuated tubes containing potassium oxalate and sodium fluoride (Sherwood Medical, St. Louis, MO).

At 1430 on d 26, horses were fitted with 14-gauge jugular catheters to characterize the plasma metabolic and hormonal responses to feeding (d 27, which included the two meals), and to an i.v. epinephrine challenge followed by an IVGTT (d 29). Blood was drawn hourly for 14 h beginning at 0600 on d 27; this period was from 2 h before the morning feeding to 4 h after the afternoon feeding. Mares were given 2 h for completion of their normal daily meals and then were allowed access to water. Only one horse failed to consume the majority of the allotted meal.

For the epinephrine challenge on d 29, blood was drawn at 15-min intervals beginning at 0800 (16 h after feeding) for 1 h, and then epinephrine HCl (Sigma Chemical, St. Louis, MO) was administered ($5 \mu\text{g/kg}$ of BW; 1 mg/mL solution in sterile saline) i.v. through the catheters. Blood was drawn at 10-min intervals for the first 60 min and then at 15-min intervals for the next 60 min. Feed and water

were withheld during the epinephrine challenge. Following a 2 h rest during which horses had access to water, two blood samples were collected 10 min apart and then glucose was administered i.v. (200 mg/kg of BW; 50% w/v solution in sterile saline). Samples of blood were drawn at 5, 10, 15, 20, 25, 30, 45, 60, 90, 120, 150, and 180 min after administration of glucose. Samples drawn on d 27 and 29 were 7 mL in volume and were placed into evacuated tubes containing potassium oxalate and sodium fluoride. Catheters were maintained with 4% sodium citrate in sterile saline.

Blood Processing and Analyses. Blood samples were placed in a 5°C refrigerator immediately after withdrawal and were centrifuged within 1 h at 1500 x g for 15 min. Plasma was harvested and stored at -15°C. Commercial kits were used for the colorimetric determinations of plasma glucose (Sigma Tech. Bull. No. 315, Sigma Chemical) and NEFA (NEFA-C kit, Wako Chemicals USA, Richmond, VA). Plasma insulin and urea N were analyzed as previously described (DePew et al., 1994). Dietary CP was determined by the Kjeldahl procedure (AOAC, 1984) and NDF and ADF by the method of Goering and Van Soest (1970).

Calculations and Statistical Procedures. Basal glucose and insulin concentrations were computed as the average of the two samples taken immediately prior to glucose administration. Clearance rates and half-lives were computed for each animal using data from 15 to 45 (glucose) and 10 to 25 min (insulin) after glucose administration. Responses of plasma glucose and insulin to IVGTT were also evaluated by computing the net areas under the response curves by summing

the time x concentration increments after subtraction of that animal's pretreatment average. For the epinephrine challenge, the net increases in glucose and NEFA concentrations over basal concentrations (average of the five, 15-min plasma samples) were calculated to adjust for variation among groups before the challenge. Net areas under the NEFA response curves were also calculated as described above.

Statistical analyses were conducted using the GLM procedure of SAS (1988). A completely randomized design with a 2 x 2 factorial arrangement of treatments tested protein restriction, energy restriction, and their interaction using the residual error. For samples taken over time, multivariate repeated measures analysis included testing time, time x protein, time x energy, and time x protein x energy. For most variables, the four statistics given in multivariate analysis were identical; however, Wilks' Lambda probability was reported. Where an insufficient number of degrees of freedom occurred (number of repeated measures exceeded error degrees of freedom), the Greenhouse-Geisser adjusted probability was used (SAS, 1988).

Results

Intake and Weight Response. All mares consumed their allotted meal within 2 h, with the exception of one mare fed control protein and energy that periodically required the full feeding period for consumption, with minor feed refusal. One mare fed control protein and restricted energy was pregnant and was removed from

the experiment. During the first weighing period (9 d), mares fed restricted energy diets lost .37 kg/d, while those fed control energy gained .19 kg/d (energy effect, $P = .07$; Table 3.2). Similarly, for the entire 24-d weighing period, mares consuming restricted energy diets lost more BW than those consuming control energy diets (energy effect, $P = .05$). Nevertheless, protein restriction had no influence ($P > .1$) on BW during any period of the experiment.

Daily Plasma Concentrations. Daily plasma concentrations of glucose and insulin before the morning meal were not affected by dietary protein and(or) energy levels (protein and energy effects, $P > .1$; Figure 3.1). Mares fed energy restricted diets had 2.5 times higher daily NEFA concentrations than those fed control energy (energy effect, $P = .0001$; Figure 3.1). Restricting protein lowered daily urea N by 19% (protein effect, $P = .002$; Figure 3.1), while restricting energy raised urea N by 19% (energy effect, $P = .013$). These effects of protein and(or) energy restriction occurred within 24 h of initiating dietary treatments and were consistent (day effect, $P > .1$) throughout the remaining 24 d.

Responses to Feeding. Concentrations of plasma glucose increased within 2 h after both meals on d 27 (time effect, $P = .08$; Figure 3.2); however, the response was not altered ($P > .1$) by protein and(or) energy restriction. Likewise, insulin concentrations (Figure 3.2) peaked approximately 2 h after meal initiation, returned to prefeeding levels approximately 7 h later, and peaked again 2 to 3 h after the afternoon meal (time effect, $P = .03$), but were not affected by protein and(or)

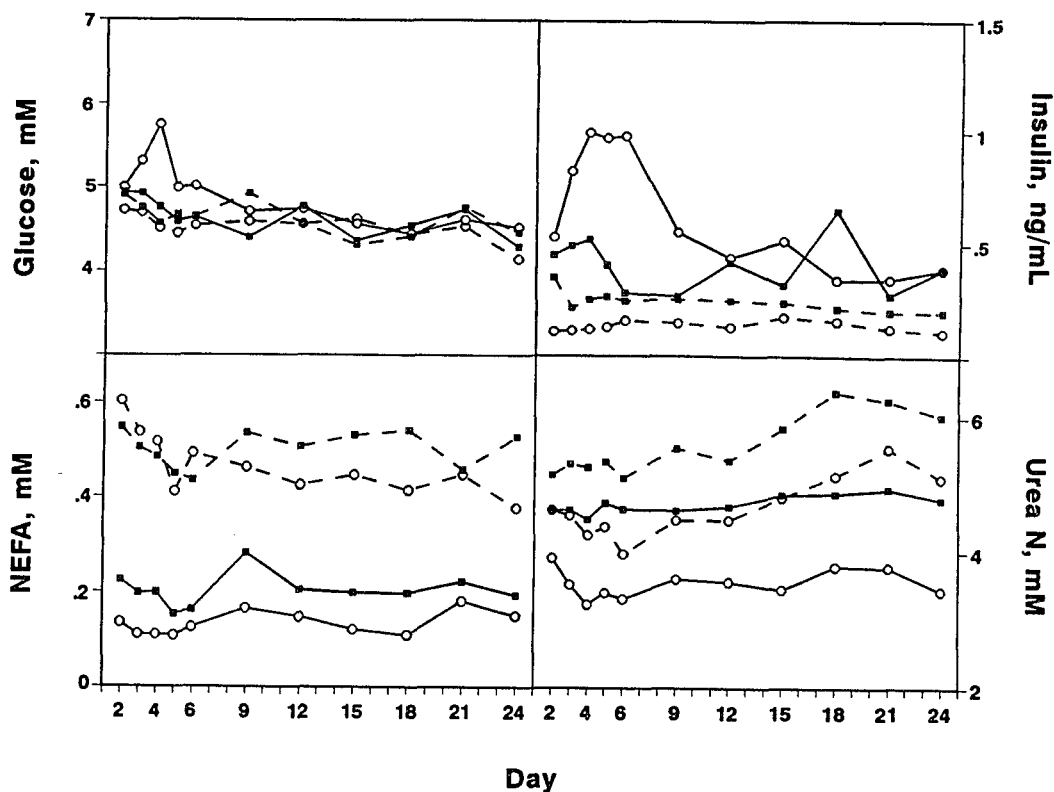


Figure 3.1. Daily concentrations of plasma glucose, NEFA, insulin, and urea N. Adult mares were fed 100 (control) or 50% (restricted) of protein and(or) energy requirement. Mares received their first treatment meal at 0800 on d 1. Solid line = control energy; dash line = restricted energy; ■ = control protein; ○ = restricted protein. SE were .27 mM, .04 ng/mL, .22 mM, and .27 mM for glucose, NEFA, insulin, and urea N, respectively.

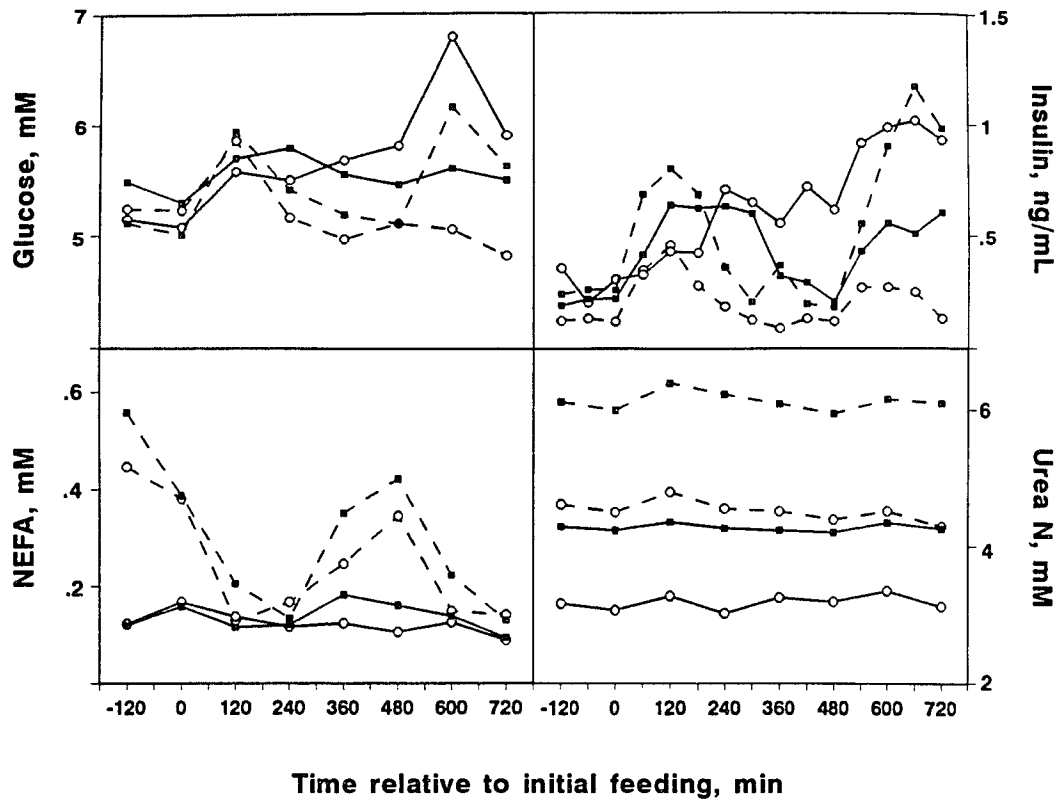


Figure 3.2. Concentrations of plasma glucose, NEFA, insulin, and urea N relative to two meals. Adult mares were fed 100 (control) or 50% (restricted) of protein and(or) energy requirement at 0 and 480 min. Solid line = control energy; dash line = restricted energy; ■ = control protein; ○ = restricted protein. SE were .42 mM, .04 ng/mL, .25 mM, and .22 mM for glucose, NEFA, insulin, and urea N, respectively.

energy restriction. This pattern of plasma insulin response to feeding was similar for all treatments except the control energy, restricted protein treatment which contained one mare who did not consume the entire meal. This mare's plasma glucose and insulin concentrations continually increased during the feeding period.

Plasma NEFA concentrations (Figure 3.2) did not change after feeding in mares fed control energy but decreased after both meals in mares fed energy restricted diets (energy x time interaction, $P = .005$). Plasma NEFA concentrations were not influenced by protein restriction (protein effect, $P = .42$), but were elevated in mares fed restricted energy (energy effect, $P = .0005$). Across all treatments, plasma urea N increased after both meals (time effect, $P = .002$; Figure 3.2). Protein restriction decreased (protein effect, $P = .008$) and energy restriction increased urea N concentrations (energy effect, $P = .002$).

Intravenous Glucose Tolerance Test. Basal concentrations of glucose and insulin prior to the IVGTT were not affected ($P > .1$) by dietary protein or energy (Table 3.3 and Figure 3.3). Clearance rate and half-life of glucose did not differ ($P > .1$) with protein or energy restriction, whereas the plasma glucose response (area under the curve) was reduced by 26% in mares fed restricted protein (protein effect, $P = .04$) and increased by 54% in mares fed restricted energy (energy effect, $P = .009$; Table 3.3). Plasma insulin concentrations (Figure 3.3) remained low following glucose administration in mares fed restricted protein and energy (average .32 ng/mL), whereas mares on the other diets averaged 1.11 ng/mL (diet effect; $P = .0001$). Clearance rate of insulin (Table 3.3) during the IVGTT was 63% greater

Table 3.3. Kinetic parameters during an i.v. glucose tolerance test and epinephrine challenge. Adult mares were fed 100 (control) or 50% (restricted) of protein and(or) energy requirement

Item	Control protein		Restricted protein		SE
	Control Energy	Restricted Energy	Control Energy	Restricted Energy	
n	4	3	4	4	
Glucose tolerance test ^a					
Glucose kinetics					
Basal concentration, mM	4.75	4.63	5.15	5.22	.38
Clearance rate, %/min	.85	.81	1.36	.84	.19
Half-life, min	82	89	60	87	10
Curve area, min•mM ^b	404	595	264	453	58
Insulin kinetics					
Basal concentrations, ng/mL	.17	.16	1.01	.08	.45
Clearance rate, %/min ^d	1.71	3.31	.69	5.39	.56
Half-life, min ^e	43	23	145	14	33
Curve area, min•ng/mL ^c	122	137	74	46	30
Epinephrine Challenge					
Glucose curve area, min•mM	59	78	132	68	24
NEFA curve area, min•mM	9.95	8.09	9.03	4.67	3.21

^aBasal glucose and insulin concentrations were taken immediately prior to glucose challenge; glucose and insulin clearance rates and half-lives were computed from 15 to 45 and 10 to 25 min post-challenge, respectively.

^bProtein effect (P=.04) and energy effect (P=.009).

^cProtein effect (P=.05).

^dEnergy effect (P=.0002) and protein x energy interaction (P=.02).

^eEnergy effect (P=.05).

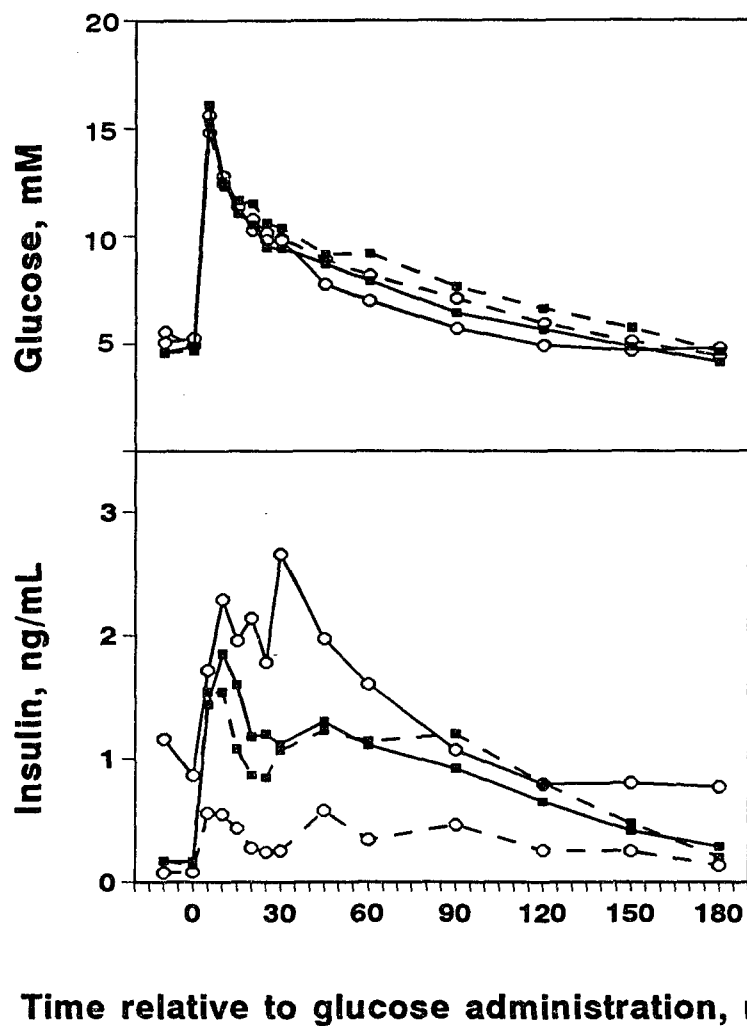


Figure 3.3. Concentrations of plasma glucose and insulin relative to an i.v. glucose tolerance test. Adult mares were fed 100 (control) or 50% (restricted) of protein and/or energy requirement. Solid line = control energy; dash line = restricted energy; ■ = control protein; ○ = restricted protein.

in mares fed restricted as compared to control protein in combination with restricted energy, but was 60% less in mares fed restricted as compared to control protein with control energy levels (protein x energy interaction, $P = .02$). The half-life of insulin (Table 3.3) was reduced in mares fed restricted energy (energy effect, $P = .05$). The plasma insulin response (area under the curve; Table 3.3) was reduced in protein restricted mares (protein effect, $P = .05$). Insulin responded similarly in all mares with the exception of one horse fed control energy and restricted protein which had a baseline insulin concentration of 3.05 ng/mL, whereas the remaining mares averaged .16 ng/mL.

Epinephrine Challenge. Prior to the administration of epinephrine, plasma NEFA concentrations averaged .15 and .28 mM ($P = .007$), while glucose concentrations averaged 4.9 and 4.8 mM ($P = .79$), for mares fed control and restricted energy, respectively (Figure 3.4). Protein restriction did not affect the plasma NEFA response to epinephrine administration. However, energy restriction increased the initial magnitude, but after 50 min, reduced plasma NEFA below pre-epinephrine administration concentrations (energy x time interaction, $P = .06$). Restriction of protein and(or) energy did not influence the glucose or NEFA response (areas under the curve) following the epinephrine challenge (Table 3.3).

Discussion

Changes in plasma NEFA and urea N concentrations were apparent within 24 h after alterations in dietary protein and(or) energy levels and further changes were

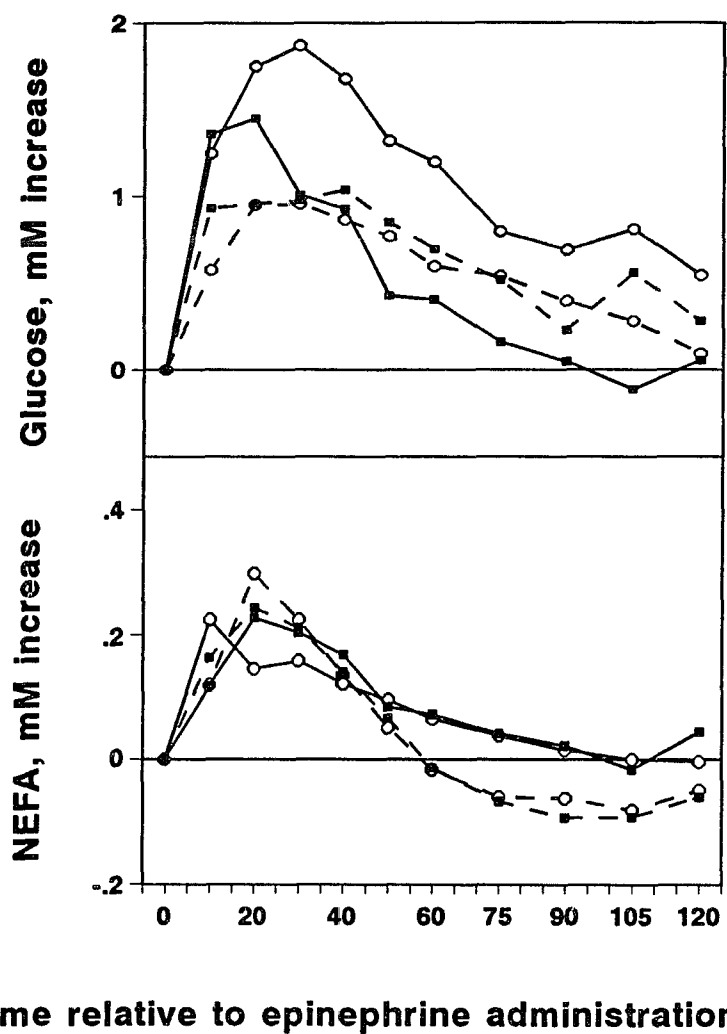


Figure 3.4. Increase above pretreatment concentrations of plasma glucose and NEFA concentrations relative to an epinephrine challenge. Adult mares were fed 100 (control) or 50% (restricted) of protein and(or) energy requirement. Solid line = control energy; dash line = restricted energy; ■ = control protein; ○ = restricted protein. Mean pretreatment concentrations of NEFA were .15 and .28 mM, while glucose were 4.9 and 4.8 mM for control and restricted energy, respectively.

not observed throughout the remaining 33 d. Because all mares were being maintained on pasture and fed similarly prior to onset of dietary treatments, it is assumed that they all had similar starting concentrations of these metabolites. In support of this assumption, others have observed immediate metabolic and hormonal responses to restriction and refeeding in sheep (Blum et al., 1980) and steers (Blum et al., 1985). Due to the dramatic effects observed within the first 24 h, further research is warranted to characterize the short-term changes (first 24 h) in these plasma constituents to dietary protein and energy restriction in the horse.

Daily concentrations of plasma glucose and insulin in these mares before feeding (16 h after previous meal) were similar to previously reported values for unfed geldings 2 yrs of age (Stull and Rodiek, 1988), adult mares and stallions (DePew et al., 1994), and ponies (Freestone et al., 1991). Dietary protein and(or) energy restriction did not influence prefeeding plasma glucose or insulin concentrations. This was also observed in weanling Thoroughbreds fed 80% of their protein and energy requirements by Glade et al. (1984). Prunier et al. (1993) also reported no variations in plasma glucose and insulin concentrations in peripubertal gilts fed near or 50% of ad libitum intake. In contrast to studies with nonruminants, plasma glucose and insulin concentrations were lower prior to feeding in steers restricted to intakes approximately half of the levels fed to control steers (Blum et al., 1985). In addition, insulin was positively correlated to protein and energy, or DM intake in young sheep (Bassett et al., 1971; Waghorn et al., 1987) and was positively correlated to protein intake in young bulls fed isocaloric

diets (Martin et al., 1979). However, plasma insulin concentrations were not affected in lactating dairy cows fed isocaloric diets at either 80 or 100% of CP requirements (Chew et al., 1984a).

Protein and(or) energy restriction also did not influence concentrations of plasma glucose and insulin after the two meals on d 27. Plasma glucose concentrations increased within 1 to 2 h after each meal and returned to prefeeding levels within 5 to 6 h. Similar responses in insulin concentrations were noted previously in horses by Glade et al. (1984), Stull and Rodiek (1988), and DePew et al. (1994). Horses and pigs respond similarly after feeding; for example, the rises in glucose and insulin concentrations were similar in pigs after feeding regardless of intake (Prunier et al., 1993). However, ruminants exhibit a slow rise in plasma glucose concentrations after feeding, peaking at 6 to 8 h (McCarthy et al., 1992). Hepatic gluconeogenesis, following absorption of propionate, lactate, and amino acids (Herdt, 1988), provides this delayed elevation in ruminants, whereas in horses, direct glucose absorption increases plasma concentrations within 1 h following feeding.

Plasma glucose concentrations returned to baseline values sooner after glucose administration in mares fed control energy, as indicated by the areas under the response curve. This may indicate that the tissues of mares fed control energy levels are less adapted to glucose conservation, as suggested by Waghorn et al. (1987). It is also feasible that slightly higher insulin concentrations in mares fed control energy aided the more rapid glucose decline or that the tissues might have

been more responsive to the uptake of glucose. Even though initial clearance rates were not influenced by diet, Cole et al. (1993) noted that glucose fractional removal rates were faster (calculated between 5 and 60 min after administration) in fed than in unfed (3 d) lambs. A more rapid decline in plasma glucose and insulin concentrations after glucose infusion was also observed in yearling sheep at higher feed intakes (Waghorn et al., 1987). Restriction of protein in the present experiment also resulted in a more rapid return of normal plasma glucose concentrations at both energy levels. Glucose metabolism in horses is more comparable to ruminants when fed high forage diets, but more comparable to the nonruminant pattern when fed high grain diets (Argenzio and Hintz, 1972).

Restricting protein and energy had varying effects on the insulin response to glucose administration. Energy restriction increased insulin half-life, while protein restriction decreased the insulin response (area under the curve). The low plasma insulin response to glucose administration in mares fed restricted protein and energy was similar to observations noted by Garcia and Beech (1986), where insulin response to glucose was lower in adult horses fed a mixture of timothy and alfalfa hay ad libitum as compared to those fed the hay plus a grain supplement. It is possible that combined restriction of protein and energy either decreased insulin production or secretion, decreased beta cell sensitivity to glucose, or as suggested by Bergman (1989), decreased tissue glucose transporters. This response was not observed with either protein or energy restriction alone.

Restriction of energy supplying nutrients would be expected to reduce glycogen stores. Hence, induction of hepatic glycogenolysis with exogenous epinephrine would be expected to result in smaller increases in plasma glucose in restricted animals. Indeed, 3 or 4 d of feed deprivation reduced the glucose response to epinephrine infusions in cattle (Blum et al., 1982; Frohli and Blum, 1988). However, in the present experiment, plasma glucose concentrations responded similarly to epinephrine administration regardless of protein or energy intake.

Plasma urea N increases as dietary protein increases in horses (Fonnesbeck and Symons, 1969; Patterson et al., 1985), ruminants (Preston et al., 1965; Hammond, 1983), and other nonruminants (Eggum, 1970). However, in ruminants, increased energy intake, with isonitrogenous diets, decreased plasma urea N concentrations (Hammond, 1983). The increase in urea N concentrations due to energy restriction in these mares is consistent with previous reports. Likewise, the response of urea N to restriction of protein followed previously reported observations during low protein intakes in lambs (Preston et al., 1965) and pigs (Eggum, 1970). Similar dietary protein and energy ratios appear to provide comparable plasma urea N concentrations, regardless of intake. In contrast, a reduction in either protein or energy alone alters plasma urea N concentrations. The elevated plasma urea N concentrations during energy restriction are presumably a consequence of increased use of protein for energy, and reduced plasma urea N concentrations during protein restriction likely arise from increased protein conservation. These measurements

provide a reference for the use of plasma urea N concentrations to determine imbalances of dietary protein or energy in the horse.

In cattle, small but significant effects of feeding on plasma urea N concentrations were frequently observed (Manston et al., 1981; Hammond, 1983). However, in pigs, a more marked increase in plasma urea N occurred in response to feeding (Eggum, 1970). Horses seem to more closely resemble ruminants than nonruminants with minor but significant increases in plasma urea N associated with feeding. This phenomenon was also reported by Depew et al. (1993). In contrast, when sampling every 90 min, Hintz and Schryver (1972) found no change in plasma urea N concentrations in ponies after feeding. Current practices of feeding swine to appropriately meet their amino acid requirements, resulting in lower plasma urea N as compared to horses fed to meet CP requirements, likely explains the variations of plasma urea N during feeding between the two species.

Energy intakes were inversely related to NEFA concentrations in cattle (Holmes and Lambourne, 1970). In the present experiment, NEFA concentrations were elevated in mares on restricted energy, even though insulin concentrations in daily samples and after feeding did not differ among treatments. In humans on restricted energy intake, the combination of reduced insulin concentrations and increased growth hormone and epinephrine concentrations result in fatty acid mobilization from adipose tissue (Guyton, 1991).

In mares fed restricted energy, NEFA concentrations decreased with feeding, likely a result of temporal reductions in fatty acid mobilization, due to greater

circulating insulin concentrations, in addition to immediate dietary fatty acid utilization. However, feeding did not influence NEFA concentrations in mares fed control energy, even though insulin was similar between the two energy levels. Low NEFA concentrations in mares fed control energy suggest little lipolytic activity was occurring between meals; therefore, the elevated insulin concentrations had less of an effect on NEFA concentrations after feeding. Similar results have been reported by Prunier et al. (1993), who fed gilts at levels close to ad libitum or restricted to half ad libitum intake. In both groups, plasma free fatty acids were reduced after feeding (16 h after previous meal) at 160 d of age, while at 210 d of age, during which control gilts were potentially consuming intakes more closely matching their requirements, only restricted gilts had lower plasma free fatty acids following feeding. In the horse, it appears that plasma NEFA concentration is an excellent indicator of energy status; however, time relative to feeding must be taken into consideration.

During 3 to 4 d of feed deprivation, Blum et al. (1982) and Frohli and Blum (1988) reported increased NEFA responses to epinephrine infusions in cattle. In the present experiment, similar responses to dietary restriction were not observed during the epinephrine challenge; however, in mares fed restricted energy, a greater initial response followed by reductions below initial concentrations were noted. This more rapid increase in NEFA concentrations likely indicates that these energy restricted mares had a more responsive lipolytic system and had adjusted to energy restriction by more efficiently utilizing circulating fatty acids.

In conclusion, restriction of protein and(or) energy altered plasma NEFA and urea N concentrations within 24 h of initiation of dietary treatments. Plasma glucose and insulin concentrations were not influenced by protein and(or) energy restriction before or after feeding, but varied with glucose administration. Energy restriction altered the plasma NEFA response to feeding and epinephrine administration.

Implications

Several plasma constituents are altered by protein and(or) energy restriction during the feeding period, glucose tolerance test, and epinephrine challenge in mares. Rapid (within 24 h) metabolic and hormonal responses occur with dietary alterations. Plasma constituent variations in horses during feeding appear similar to swine, with the exception of urea, where horses more closely resemble ruminants. These results emphasize the importance of specific diet formulation in relation to NRC requirements when monitoring plasma metabolites and hormones and provide a basis for determining nutritional status from plasma measurements.

CHAPTER IV

DIETARY PROTEIN AND(OR) ENERGY RESTRICTION IN MARES: PLASMA PROLACTIN, IGF-1, CORTISOL, THYROID HORMONE, AND GROWTH HORMONE RESPONSES TO FEEDING, GLUCOSE, AND EPINEPHRINE

Introduction

A more complete description of the responses of circulating hormones to alterations in dietary protein and(or) intakes is necessary to describe the significance of appropriate and well defined dietary treatments in endocrine studies and also to provide reference information related to hormones not routinely measured in the horse. Plasma IGF-1 (Trembly et al., 1993) and GH (Thompson et al., 1992; Stewart et al., 1993; DePew et al., 1994) data was characterized in horses of different age, sex, or breed. Plasma prolactin was reported to increase relative to feeding in mares and stallions (DePew et al., 1994). Glade et al. (1984) and Glade and Reimers (1985) reported no differences in plasma T_3 and T_4 in weanlings fed restricted diets. In the horse, it remains unclear how nutrition influences many endocrine responses. In ruminants, plasma thyroid hormone concentrations are diminished with reduced intake (Blum et al., 1985; Ellenberger et al., 1989). Reduced plasma IGF-1 concentrations were associated with

nutritional deficiencies in heifers (Houseknecht et al., 1988; Granger et al., 1989). Nutrient restriction decreased, did not change, or increased cortisol concentrations in lambs (Ghani, 1988), cows (Schrack et al., 1990), and gilts (Pruiner et al., 1993), respectively. In ruminants, dietary protein and(or) energy restriction elevated circulating GH (deBoer et al., 1985; Granger et al., 1989; Thomas et al., 1990), whereas in rats restriction reduced plasma GH (Schalch and Cree, 1985; Achilles et al., 1982). Because of varying reports of the endocrine responses to dietary restriction and of the unclear specific nutrient involvement, further research is warranted in other species, as well in the horse. Therefore, the objective of this experiment was to characterize the effects of protein and(or) energy restriction on plasma IGF-1, cortisol, T_3 , T_4 , GH, and prolactin concentrations on a daily basis, after feeding, and after i.v. administration of glucose or epinephrine.

Experimental Procedures

Animals and Dietary Treatments. Sixteen light horse mares (8 to 9 yr of age; 457 to 579 kg initial BW), grazing native grass pasture, were stratified by weight (4 horses/treatment) to one of four dietary treatments in a completely randomized design with a 2 x 2 factorial arrangement of treatments. Dietary treatments were formulated with the aim of meeting either 100 (control) or 50% (restricted) of the CP and DE requirement for maintenance (Table 3.1; NRC, 1989); actual intakes averaged 98 and 55% (Table 3.2). The experiment was conducted during the months of November and December, 1992. Horses were offered Alicia

bermudagrass hay at .6 kg of DM/100 kg of BW, which provided 30% of the CP requirement in the control diet. The bermudagrass hay (DM basis) contained 6.5% CP, 45.2% ADF, 65.0% NDF, and was calculated to contain 1.78 Mcal DE/kg (NRC, 1982). Supplements were formulated and added in varying amounts (.6 and .22 kg of DM/100 kg of BW for control and restricted energy, respectively) to provide the appropriate amount of CP and DE (Tables 3.1 and 3.2). Supplements were formulated to provide similar amounts of cottonseed hulls and to at least meet the requirements for Ca, P, Mg, Na, K, and S. Before initiation of the experiment, horses were adapted in the pasture to the hay for 10 d and to the control supplement for 5 d. During the experiment, horses were housed in covered stalls where they were offered their ration of hay and supplement in two equal portions at 0800 and 1600 and had continual access to water.

Blood Sampling. Metabolic status was monitored by measurement of plasma IGF-1, cortisol, T_3 , T_4 , and prolactin daily for 5 d beginning on d 2, and then every third day after d 6. Blood (7 mL) was drawn at 0800 (16 h after feeding) by jugular venipuncture into evacuated tubes containing potassium oxalate and sodium fluoride (Sherwood Medical, St. Louis, MO).

At 1430 on d 26, horses were fitted with 14-gauge jugular catheters to characterize the prolactin and GH responses to feeding (d 27, which included the two meals), and to an i.v. epinephrine challenge followed by an IVGTT (d 29). For the IVGTT and epinephrine challenge, prolactin and GH, respectively, were monitored. Blood was drawn every 15 min for 14 h beginning at 0600 on d 27; this

period was from 2 h before the morning feeding to 4 h after the afternoon feeding. Mares were given 2 h for completion of their normal daily meals and then were allowed access to water. Only one horse failed to consume the majority of the allotted meal.

For the epinephrine challenge on d 29, blood was drawn at 15-min intervals beginning at 0800 (16 h after feeding) for 1 h, and then epinephrine HCl (Sigma Chemical, St. Louis, MO) was administered ($5\text{ }\mu\text{g/kg}$ of BW; 1 mg/mL solution in sterile saline) i.v. through the catheters. Blood was drawn at 10-min intervals for the first 60 min and then at 15-min intervals for the next 60 min. Feed and water were withheld during the epinephrine challenge. Following a 2 h rest during which horses had access to water, two blood samples were collected 10 min apart and then glucose was administered i.v. (200 mg/kg of BW; 50% w/v solution in sterile saline). Samples of blood were drawn at 5, 10, 15, 20, 25, 30, 45, 60, 90, 120, 150, and 180 min after administration of glucose. Samples drawn on d 27 and 29 were 7 mL in volume and were placed into evacuated tubes containing potassium oxalate and sodium fluoride. Catheters were maintained with 4% sodium citrate in sterile saline.

Blood Processing and Analyses. Blood samples were placed in a 5°C refrigerator immediately after withdrawal and were centrifuged within 1 h at $1500\times g$ for 15 min. Plasma was harvested and stored at -15°C . Cortisol, IGF-1, prolactin, and GH were analyzed by RIA as previously described in horses (Thompson et al., 1988; Granger et al., 1989; Colborn et al., 1991a; Thompson et

al., 1992, respectively). Thyroid hormones were analyzed by coated-tube RIA (ICN Biochemicals, Irvine, CA). Dietary CP was determined by the Kjeldahl procedure (AOAC, 1984) and NDF and ADF by the method of Goering and Van Soest (1970).

Calculations and Statistical Procedures. Although graphical representations are reported for the IVGTT and epinephrine challenge, only baseline concentrations and the response (area under the curve) to glucose and epinephrine administration were statistically analyzed. Basal prolactin and GH concentrations were computed as the average of the two samples taken immediately prior to glucose administration. Responses of plasma prolactin to glucose and GH to epinephrine administration were evaluated by computing the net areas under the response curves by summing the time x concentration increments for each animal after subtraction of that animal's pretreatment average.

Statistical analyses were conducted using the GLM procedure of SAS (1988). A completely randomized design with a 2 x 2 factorial arrangement of treatments tested protein restriction, energy restriction, and their interaction using the residual error. Where appropriate, diet replaced protein and energy restriction and was tested with the residual error. For samples taken over time, multivariate repeated measures analysis included testing time, time x protein, time x energy, and time x protein x energy. For most variables, the four statistics given in multivariate analysis were identical; however, Wilks' Lambda probability was reported. Where an insufficient number of degrees of freedom occurred (number of repeated

measures exceeded error degrees of freedom), the multivariate Greenhouse-Geisser adjusted probability was used (SAS, 1988). Plasma GH data were analyzed via PULSAR (Gitzen and Ramirez, 1976), with parameters set as described by Thompson et al. (1992).

Results

Daily Plasma Concentrations. Mares fed control protein and energy had higher (diet effect, $P = .0001$) plasma IGF-1 concentrations prior to the morning meal than mares fed restricted protein and(or) energy (Figure 4.1). This effect of restriction on plasma IGF-1 concentrations occurred within 24 h of initiating dietary treatments and was consistent (day effect, $P = .5$) throughout the remaining 24 d. Restricting energy decreased (energy effect, $P = .01$), whereas restricting protein did not influence (protein effect, $P = .81$), daily plasma cortisol concentrations (Figure 4.1). During the course of the experiment, plasma cortisol was similar in mares fed restricted energy, but increased in mares fed control energy (day x energy effect, $P = .0009$).

Restriction of protein and(or) energy did not influence ($P > .1$) daily plasma T_3 or T_4 concentrations (Figure 4.2). Daily plasma prolactin concentrations were low in all mares (.48 ng/mL average), and did not vary with protein or energy restriction ($P > .1$; data not shown).

Response to Feeding. Mares which consumed protein restricted diets had more GH peaks ($P = .09$; Table 4.1) than mares which were fed control protein

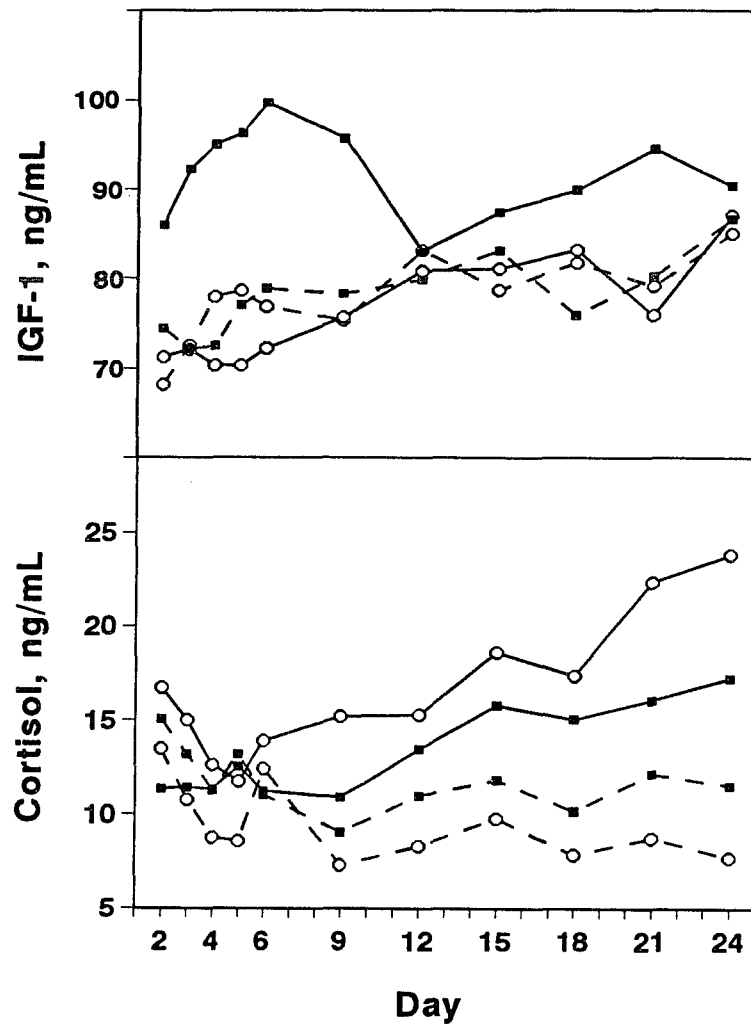


Figure 4.1. Daily concentrations of plasma IGF-1 and cortisol. Adult mares were fed 100 (control) or 50% (restricted) of protein and(or) energy requirement. Mares received their first treatment meal at 0800 on d 1. Solid line = control energy; dash line = restricted energy; ■ = control protein; ○ = restricted protein. SE were 8.7 and 1.7 ng/mL for IGF-1 and cortisol, respectively.

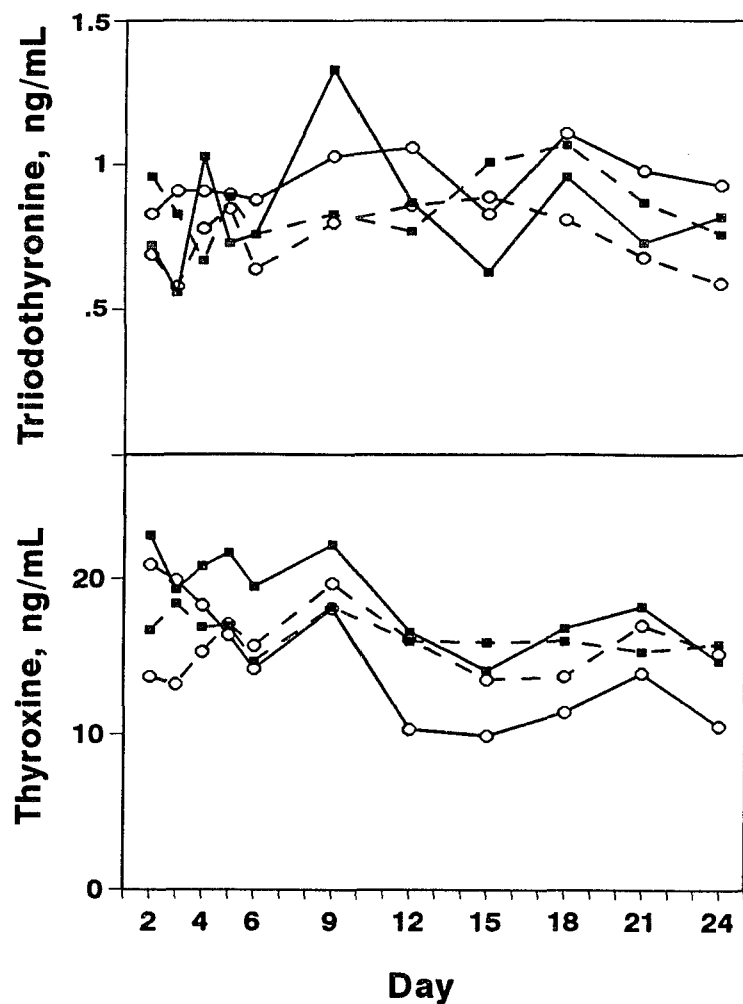


Figure 4.2. Daily concentrations of plasma triiodothyronine and thyroxine. Adult mares were fed 100 (control) or 50% (restricted) of protein and(or) energy requirement. Mares received their first treatment meal at 0800 on d 1. Solid line = control energy; dash line = restricted energy; ■ = control protein; ○ = restricted protein. SE were .02 and .22 ng/mL for triiodothyronine and thyroxine, respectively.

Table 4.1. Growth hormone secretion measurements relative to two meals.
Adult mares were fed 100 (control) or 50% (restricted)
of protein and(or) energy requirement

Item	Control protein		Restricted protein		SE
	Control	Restricted	Control	Restricted	
	Energy	Energy	Energy	Energy	
	4	3	4	4	
Baseline GH concentration, ng/mL	1.00	1.28	1.06	1.11	.18
Average GH concentration, ng/mL	1.15	1.32	1.18	1.77	.22
Number of GH peaks ^a	2.00	1.67	2.50	5.75	1.19
GH peak amplitude, ng/mL	3.51	1.41	1.46	3.15	1.13

^aProtein effect (P = .09).

diets. Protein and(or) energy restriction did not influence ($P > .1$) any other GH secretion measurements.

Feeding did not appear to have a direct influence on GH peaks as no consistent patterns were observed before or after feeding. Hematocrit was measured to insure plasma volume remained unaltered during the 14 h feeding period. Hematocrit levels did not differ ($P > .1$) with feeding or in relation to dietary treatment (Figure 4.3). Feeding increased (time effect; $P = .003$) plasma prolactin concentrations; however, this pattern was only observed in 3 of the 15 mares.

Intravenous Glucose Tolerance Test. Basal concentrations of prolactin prior to the IVGTT were not affected by protein (protein effect, $P = .73$) or energy restriction (energy effect, $P = .18$; Table 4.2 and Figure 4.4). In addition, plasma prolactin response (area under the curve) following glucose administration did not differ with protein (protein effect, $P = .68$) or energy restriction (energy effect, $P = .78$).

Epinephrine Challenge. Basal concentrations of GH prior to the epinephrine challenge were not affected by protein (protein effect, $P = .18$) or energy restriction (energy effect, $P = .96$; Table 4.2 and Figure 4.5). Even though only 4 horses responded to the epinephrine administration, mares fed control energy had a greater (energy effect, $P = .05$) plasma GH response (area under the curve).

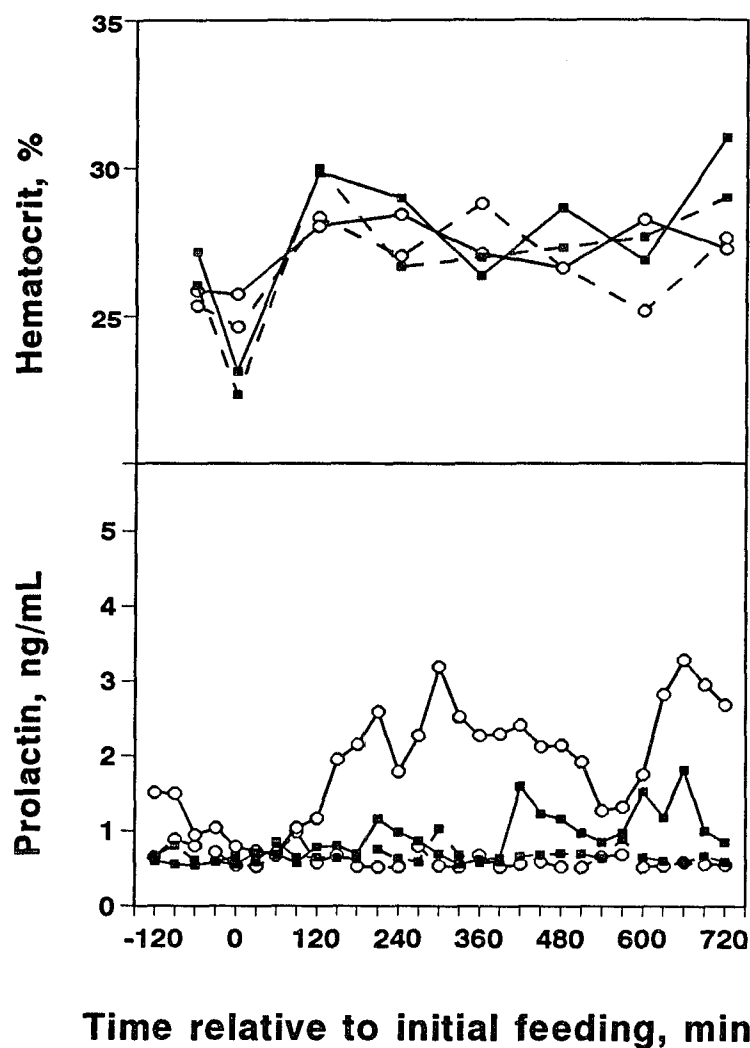


Figure 4.3. Percent of blood hematocrit and concentrations of plasma prolactin relative to two meals. Adult mares were fed 100 (control) or 50% (restricted) of protein and(or) energy requirement at 0 and 480. Solid line = control energy; dash line = restricted energy; ■ = control protein; ○ = restricted protein. SE were 1.1 % and .42 ng/mL for hematocrit and prolactin, respectively.

Table 4.2. Kinetic parameters during an i.v. glucose tolerance test and epinephrine challenge. Adult mares were fed 100 (control) or 50% (restricted) of protein and(or) energy requirement

Item	Control protein		Restricted protein		SE
	Control Energy	Restricted Energy	Control Energy	Restricted Energy	
n	4	3	4	4	
Glucose Tolerance Test					
Prolactin kinetics					
Basal concentrations, ng/mL	1.12	.345	.605	.691	.23
Curve area, min • ng/mL	-18.5	41.3	14.9	-23.5	35.6
Epinephrine Challenge					
Growth hormone kinetic					
Basal concentrations, ng/mL	1.42	1.54	1.74	1.64	.15
Curve area, min • ng/mL ^a	68.3	-10.4	141.9	-15.4	51.7

^aEnergy effect (P = .05).

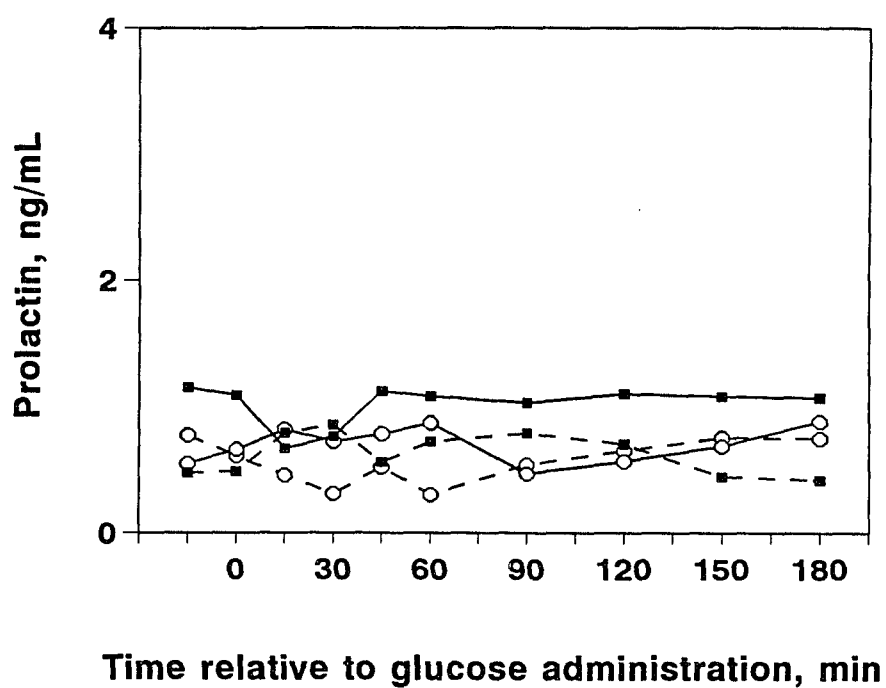


Figure 4.4. Concentrations of plasma prolactin relative to an i.v. glucose tolerance test. Adult mares were fed 100 (control) or 50% (restricted) of protein and(or) energy requirement. Solid line = control energy; dash line = restricted energy; ■ = control protein; ○ = restricted protein.

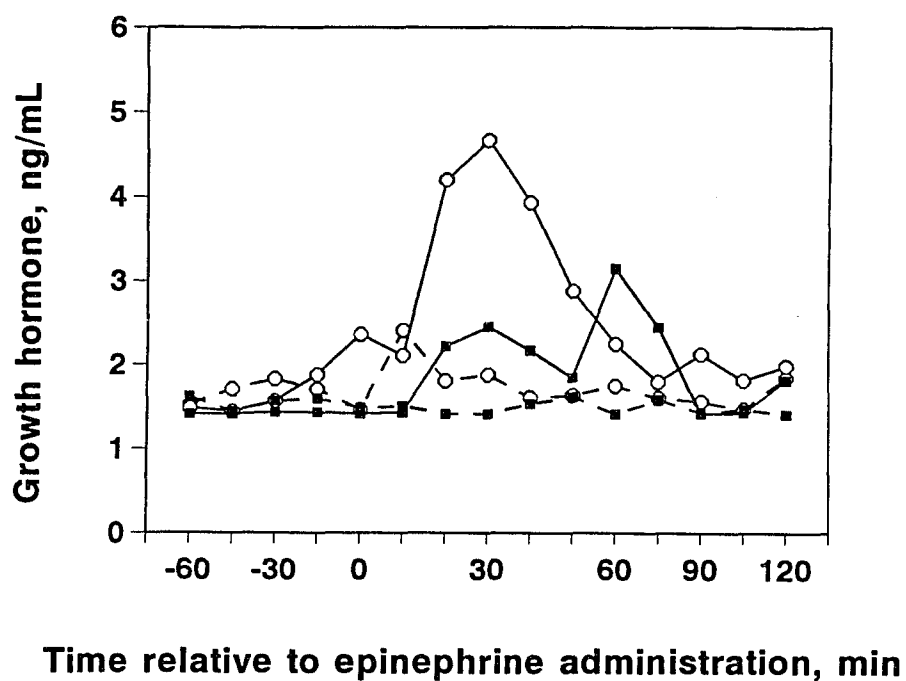


Figure 4.5. Concentrations of plasma growth hormone relative to an epinephrine challenge. Adult mares were fed 100 (control) or 50% (restricted) of protein and(or) energy requirement. Solid line = control energy; dash line = restricted energy; ■ = control protein; ○ = restricted protein.

Discussion

Reduced plasma IGF-1 concentrations have been associated with nutritional deficiencies resulting in weight loss in heifers (Houseknecht et al., 1988; Granger et al., 1989). Restriction (80%) of protein or energy requirements alone or in combination for lactating cows did not alter plasma IGF-1 concentrations (McGuire et al., 1992a). In rats, protein restriction decreased somatomedin activity and plasma concentrations (Reeves et al., 1979; Prewitt et al., 1982). It appears that in mares, either protein or energy restriction alone or in combination reduces plasma IGF-1 concentrations. In ruminants, a similar response of plasma IGF-1 concentrations to varying protein and(or) energy levels (120 and 78% of requirements) was observed during a single daily injection of bST for 4 d (McGuire et al., 1992a). In the present experiment, alterations of plasma IGF-1 concentrations occurred rapidly (within 24 h of dietary initiation). Similar short term increases in plasma IGF-1 concentrations were also observed following refeeding of feed deprived pigs (Buonomo and Baile, 1991) and humans (Clemmons and Underwood, 1991). Possible reasons for decreased plasma IGF-1 concentrations with nutritional restriction include decreased concentrations of the binding protein (McGuire et al., 1992b), affinity or number of GH receptor on the liver (Breier et al, 1988), or transcription or translation (Thissen et al., 1991). Plasma IGF-1 concentrations in these mares average 81 ng/mL, whereas plasma IGF-1 concentrations reported in foals at birth and 4 mo old averaged 219 and 210 ng/mL, respectively (Trembly et al., 1993).

Daily concentrations of plasma cortisol in these mares before feeding (16 h after previous meal) were lower (average 12.8 ng/mL) than previously reported values for horses (James et al., 1970; Glade et al., 1984; Stull and Rodiek, 1988; Sojka et al., 1993), which averaged 33 to 70 ng/mL. Restriction of energy decreased plasma cortisol concentrations during the experiment; however, concentrations increased in mares fed control energy as the experiment progressed. In cattle, acute exposure to elevated temperatures for several hours increased plasma corticosteroid concentrations, whereas prolonged exposure (24 d) decreased plasma corticosteroid concentrations (Alvarez and Johnson, 1973). Similar responses of plasma cortisol concentrations to nutrient restriction were observed in this experiment. The elevated plasma cortisol concentration in the mares fed control energy was consistent with the results of Glade et al. (1984), who reported higher plasma cortisol concentrations in weanlings fed 160 vs 80% of their protein and energy requirements (60.8 vs 48.7 ng/mL, respectively). Because of the metabolic role of cortisol, increasing gluconeogenesis, lipolysis, and protein catabolism (Guyton, 1991), it would seem that nutrient restriction would increase circulating cortisol concentrations to facilitate nutrient mobilization; however, varying results are observed. Indeed, some researchers have observed increased plasma cortisol concentrations during hypoglycemia in horses (James et al., 1970) and 5 h after feeding peripubertal gilts a diet which was 50% ad libitum intake (Pruiner et al., 1993). However, Schrick et al. (1990) reported similar daily peripheral blood cortisol concentration in cows (100 d postcalving) fed either at

high or low energy intakes. And similar to the present results, higher plasma cortisol concentrations were observed in lambs fed high as compared to moderate protein levels at two environmental temperatures (Ghani, 1988). It is feasible that immediate responses (within hours) to nutrient restriction could elevate plasma cortisol concentrations, while prolonged nutrient restriction could reduce or return cortisol to normal concentrations.

Restriction of protein and(or) energy did not influence daily plasma thyroid hormone concentrations. Others have reported decreased thyroid function with nutrient restriction. Reduced plasma T_3 and T_4 concentrations were observed with reduced feed intake in steers (Blum et al., 1985) and rats (Schalch and Cree, 1985). Likewise, Blum et al. (1980) observed reduced plasma T_3 concentrations in adult sheep fed at reduced levels, but no change in plasma T_4 concentrations, and Ellenberger et al. (1989) observed no change in plasma T_3 concentrations, but lowered plasma T_4 concentrations, in growing steers fed to gain only .37 kg/d as compared to steers fed to gain 1.4 kg/d. In horses, Glade and Reimers (1985) reported no differences in plasma T_3 or T_4 concentrations in weanling foals fed either 70, 100, or 130% of their protein and energy requirements. Glade et al. (1984) also observed similar plasma T_4 concentrations before feeding in weanlings fed either 80 or 160% of their protein and energy requirement.

Elevated circulating GH concentrations are associated with dietary protein and(or) energy restriction for the conservation of protein and carbohydrates at the expense of lipid stores (Guyton, 1991). Protein restriction increased plasma GH

concentrations in yearling rams (Barenton et al., 1987), while energy restriction increased plasma GH concentrations in growing broiler chickens (Rosebrough et al., 1989). This GH response is not evident in rats (Achilles et al., 1982; Schalch and Cree, 1985) or with mild restriction in cattle (Chew et al., 1984a; Elsasser et al., 1989). Both protein and energy restriction increased plasma GH concentrations in lactating dairy cows (deBoer et al., 1985), growing beef heifers (Granger et al., 1989), infants (Muzzo and Alcazar, 1985), and adult ewes (Thomas et al., 1990). In the present experiment, mares fed protein restricted diets had more GH episodes during the 14-h bleeding period, but this response was largely related to the increased GH episodes in mares fed both protein and energy restricted diets. It appears that in the adult mare, neither protein nor energy restriction alone increases GH as greatly as the restriction of both.

Plasma GH concentrations did not change relative to feeding and appeared random during the 14 h period. Similarly, DePew et al. (1994) reported no relationship between feeding and plasma GH concentrations. Plasma GH concentrations increased after feeding in adult dairy cows (de Boer et al., 1985) and growing lambs (Symonds et al., 1989), and decreased after feeding in adult sheep (Basset, 1974a; Trenkle, 1989) and goats (Tindal et al., 1982).

Epinephrine, which was used as a secretagogue of GH, increased GH only in four of the 15 mares, and all mares that responded were fed control energy. This is not consistent with the number of GH peaks observed during the 14-h bleeding period. Others reported that epinephrine increased plasma GH concentrations in

stallions (Thompson et al., 1992) and decreased plasma GH concentrations in yearling wethers (Bassett et al., 1971).

Thyroid function appears to have significant influences on GH action, and the uncoupling of GH and IGF-1 with nutritional deficiencies (Elsasser et al., 1989) appears to be associated with reduced plasma T_3 concentrations (Cabello and Wrutniak, 1989). The greater number of GH peaks in mares fed restricted protein and combined restricted protein and energy in addition to reduced plasma IGF-1 concentrations supports this relationship in the horse. However, the thyroid role is not evident in this experiment because all mares had similar plasma T_3 and T_4 concentrations.

Plasma prolactin concentrations are known to vary with season and temperature in cattle as well as stress in horses (Bauman et al., 1982; Colborn et al., 1991a). In horses, plasma prolactin concentration is at a minimum during the winter months (Thompson et al., 1987), which agrees with the low plasma prolactin concentrations in this experiment with subsequently no dietary influences on daily plasma prolactin concentrations. Other researchers have also reported no influences of feed restriction on plasma prolactin concentrations. Plasma prolactin concentrations were similar in weanling lambs fed either to grow normally or maintain body weight (Foster et al., 1989) and in lambs fed ad libitum or restricted intakes (Forbes et al., 1975). However, Cosgrove et al. (1991) reported elevated plasma prolactin concentrations with feed restriction (30% of ad libitum intake) as compared with ad libitum feeding in prepubertal gilts. It is unclear what influence

feed restriction has on plasma prolactin concentrations and prolactin's metabolic function during altered nutritional status.

Plasma prolactin concentrations have been shown to increase as a result of feeding in horses (DePew et al., 1994), humans (Quigley et al., 1981), goats (Bryant et al., 1970), and cattle (McAttee and Trenkle, 1971c) and have been linked to opiate peptides (Zioudrou et al., 1979), neurotransmitter precursors (Ishizuka et al., 1993), and amino acids and proteins in the meal (Carlson, 1989). It is not known whether varying dietary protein and energy levels would alter this response. Plasma prolactin concentrations increased relative to feeding in only 3 of the 15 mares in this study. The season potentially influenced this prolactin response and consequently, more information is needed to determine the varying responses of prolactin to feeding with combined alterations of diet and season in the horse.

A response of prolactin to glucose administration might indicate that the meal induced rise in plasma glucose concentrations or the associated insulin rise is related to elevated plasma prolactin concentrations following feeding. However, glucose metabolism appears to be unrelated to the feed induced prolactin response. McAtee and Trenkle (1971c) and Bryant et al. (1970) reported that glucose administration had no influences on plasma prolactin concentrations in 5 mo old heifer calves and goats, respectively. Results observed in horses appear to follow the results observed in ruminants, in that no changes in plasma prolactin concentration were observed during the IVGTT. Nonetheless, season still might have had a role in the prolactin response to the IVGTT. With feed deprived lambs

(3 d without feed), Cole et al. (1993) observed an increase in plasma prolactin concentrations in fed lambs (fed to meet requirements) following glucose administration, and showed a decline in plasma prolactin concentrations in feed-deprived (3 d) lambs following glucose administration. However, the authors suggested that the prolactin response to glucose injection might have resulted from possible elevated β -endorphin release during the stress of feed deprivation which could mediate the initial increases in prolactin.

In summary, restriction of protein and (or) energy reduced plasma IGF-1 concentrations within 24 h of initiation of dietary treatments. Energy restriction decreased daily plasma cortisol concentrations between d 9 and the end of the study. Nutrient restriction did not influence daily plasma prolactin or thyroid hormone concentrations and plasma prolactin concentrations increased with feeding in only 3 of the 15 mares, and did not respond to glucose administration. Protein restriction increased GH episodes during a 14-h feeding period; however, energy restriction diminished the GH response to epinephrine.

Implications

This experiment provides a more complete description of the responses of circulating hormones to alterations in dietary protein and(or) intakes and documents the significance of appropriate, well controlled dietary treatments in endocrine studies. Alterations of IGF-1, cortisol, and GH concentrations during protein and(or) energy restriction highlight the metabolic significance of diet

formulation and nutrition on endocrine studies. Rapid (within 24 h) alterations of IGF-1 occur with nutrient restriction provide evidence for the importance of early sampling during dietary manipulation. This experiment provides reference information relative to hormones not routinely measured in the horse.

CHAPTER V

DIETARY PROTEIN AND ENERGY RESTRICTION IN MARES: EVIDENCE OF RAPID CHANGES OF PLASMA METABOLIC AND HORMONAL CONCENTRATIONS DURING DIETARY ALTERATION

Introduction

In the first experiment (Chapters III and IV), mares were fed either control or restricted levels of their protein and(or) energy requirements with no initial controlled feeding period, and therefore no pretreatment samples were collected. Within the first 24 h after dietary initiation, alterations were already observed for plasma NEFA, urea N, and IGF-1 concentrations. Rapid alterations (within days) of plasma glucose, NEFA, and urea N concentrations to dietary restriction, feed deprivation, and refeeding were also observed in ruminants (Blum et al., 1985; Cole et al., 1988; Ellenberger et al., 1989). Immediate increases of plasma IGF-1 concentrations were reported after refeeding following feed deprivation in pigs (Buonomo and Baile, 1991) and humans (Clemmons and Underwood, 1991). Plasma thyroid hormones also responded within days after feed intake was reduced in steers (Blum et al., 1985; Ellenberger et al., 1989) and rats (Schalch and Cree, 1985). A complete description of immediate responses (within hours) of meal alterations has not been demonstrated. Therefore the objective of this experiment was to document immediate responses of plasma glucose, NEFA, urea N, insulin,

glucagon, IGF-1, cortisol, and T_4 concentrations during dietary alterations to restricted protein and energy intakes and then during refeeding of control intakes.

Experimental Procedures

Animals and Dietary Treatments. Twelve light horse mares (8 to 9 yr of age; 436 to 630 kg initial BW) grazing native grass pasture were stratified by weight (6 horses/treatment) to one of two dietary treatments in a completely randomized design. Dietary treatments were formulated with the aim of meeting either 100 (control) or 50% (restricted) of the CP and DE requirement for maintenance (Table 3.1; NRC, 1989); actual intakes averaged 98 and 55% (Table 5.1). The experiment was conducted during October, 1993. Horses were offered Alicia bermuda grass hay at .6 kg of DM/100 kg of BW, which provided 30% of the CP requirement in the control diet. The bermuda grass hay (DM basis) analyzed 6.5% CP, 45.2% ADF, 65.0% NDF and was calculated to contain 1.78 Mcal DE/kg (NRC, 1982). Supplements were formulated and added in varying levels (.6 and .22 kg of DM/100 kg of BW for control and restricted diet, respectively) to provide the appropriate amount of CP and DE (Tables 3.1 and 5.1). Supplements were formulated to provide similar amounts of cottonseed hulls and to at least meet the requirements Ca, P, Mg, Na, K, and S. Before initiation of the experiment, horses were adapted in the pasture to the hay for 10 d and to the control supplement for 5 d. All mares consumed the control diet from d 1 to d 7. On d 8, half of the mares (restricted treatment) were switched to the restricted diet at 0800, consumed

Table 5.1. Daily intake of mares. Adult mares were fed 100 (control) or 50% (restricted) of protein and energy requirement

Item	Control	Restricted
n	6	6
Initial BW, kg	526	525
Intake		
DM, kg/d		
Hay	3.15	3.15
Supplement	3.15	1.16
CP, g/d		
Hay	208	208
Supplement	489	158
DE, Mcal/d		
Hay	5.61	5.61
Supplement	11.04	3.70

that diet for 7 d, and then were returned to the control diet on d 15. The remaining mares (control treatment) consumed the control diet during the entire experiment. Horses were housed in stalls during the entire study, offered equal portions of ration at 0800 and 1600, and allowed continual access to water.

Blood Sampling, Processing, and Analysis. Blood (7 mL) was drawn at 0700 (15 h after the afternoon feeding) on d 6, 7, 10, 11, 12, 13, and 14 by jugular venipuncture into two evacuated tubes, one containing potassium oxalate and sodium fluoride for analysis of glucose, NEFA, and urea N, and the other containing K₃EDTA (Sherwood Medical, St. Louis, MO) for analysis of IGF-1, cortisol, and T₄. A 3-mL aliquot of blood was taken from the K₃EDTA tubes and added to 37.5 μ L of benzamidine HCl (protease inhibitor, 2.401 M solution) for analysis of insulin and glucagon.

On d 8 and 15 of the experiment at 0600, horses were fitted with 14-ga indwelling jugular catheters. Blood was drawn from 0700 (15 h after the last afternoon feeding) through 2000 (4 h after the afternoon feeding) at hourly intervals, then every 2 h until 0800 on d 9. During bleeding periods, the supplement was given 30 min prior to the hay. Mares were allowed 3 h to consume the morning meal, and any remaining feed from the morning meal was added to the afternoon meal; they were then allowed sufficient time to complete the entire daily ration. All horses were allowed free access to water during the bleeding periods. At the 2 h blood sampling times, blood (14 mL) was drawn and divided in two evacuated tubes, one containing potassium oxalate and sodium fluoride for analysis

of glucose, NEFA, and urea N, and one containing K₃EDTA for IGF-1, and T₄. During the hourly sampling times, an additional 7 mL of blood was drawn and placed in evacuated tubes containing K₃EDTA and a 3-mL aliquot of blood was added to 37.5 μ L of benzamidine HCl (protease inhibitor, 2.401 M solution) for analysis of insulin and glucagon.

Blood Processing and Analyses. Blood samples were placed in a 5°C refrigerator immediately after withdrawal and were centrifuged within 1 h at 1500 x g for 15 min. Plasma was harvested and stored at -15°C. Commercial kits were used in the colorimetric determinations of plasma glucose (Sigma Tech. Bull. No. 315, Sigma Chemical), and NEFA (NEFA-C kit, Wako Chemicals USA, Richmond, VA), and for the RIA of T₄ (ICN Biochemicals, Irvine, CA). Plasma urea N, insulin, glucagon (DePew et al., 1994), and IGF-1 (Granger et al., 1989) were analyzed as previously described. Dietary CP was determined by the Kjeldahl procedure (AOAC, 1984) and NDF and ADF by the method of Goering and VanSoest (1970).

Statistical Procedures. Statistical analyses were conducted using the GLM procedure of SAS (1988). Blood samples taken on d 6 and 7 were averaged and analyzed to determine differences during the control feeding period. Three analyses were conducted which included the daily sampling period (d 6, 7, 10, 11, 12, 13, and 14), and the two 24-h periods of dietary alterations. A completely randomized design tested dietary treatment with the residual error and the multivariate repeated measures analysis included testing time and time x diet.

Wilks' Lambda probability was reported; when an insufficient degrees of freedom occurred (number of repeated measures exceeded error df), the multivariate Greenhouse-Geisser adjusted probability was used (SAS, 1988).

Results

Plasma Glucose, NEFA and Urea N Responses. Mares had similar plasma glucose concentrations during the control feeding period ($P = .23$), whereas restriction of protein and energy reduced (diet effect, $P = .01$) mean plasma glucose concentrations during the daily sampling period (Figure 5.1). Mares which were switched to restricted intakes had a diminished (diet effect, $P = .005$) plasma glucose feeding response during the 24 h dietary alteration period which lead to no influences of feeding on plasma glucose concentrations (time effect, $P = .31$). However, when restricted mares were returned to the control diet, their plasma glucose concentrations were similar to mares fed the control diet (diet effect, $P = .78$) and subsequently both meals increased plasma glucose concentrations (time effect, $P = .04$).

Plasma NEFA concentrations (Figure 5.2) were similar ($P = .44$) in all mares before initiation of dietary treatments; however, they increased during protein and energy restriction (day x diet interaction, $P = .0001$). During the initial 8 h following consumption of the first restricted meal, plasma NEFA concentration in these mares began to rise, with a continued elevation beginning 4 h following the afternoon meal; control mares remained consistent throughout the 24 h period

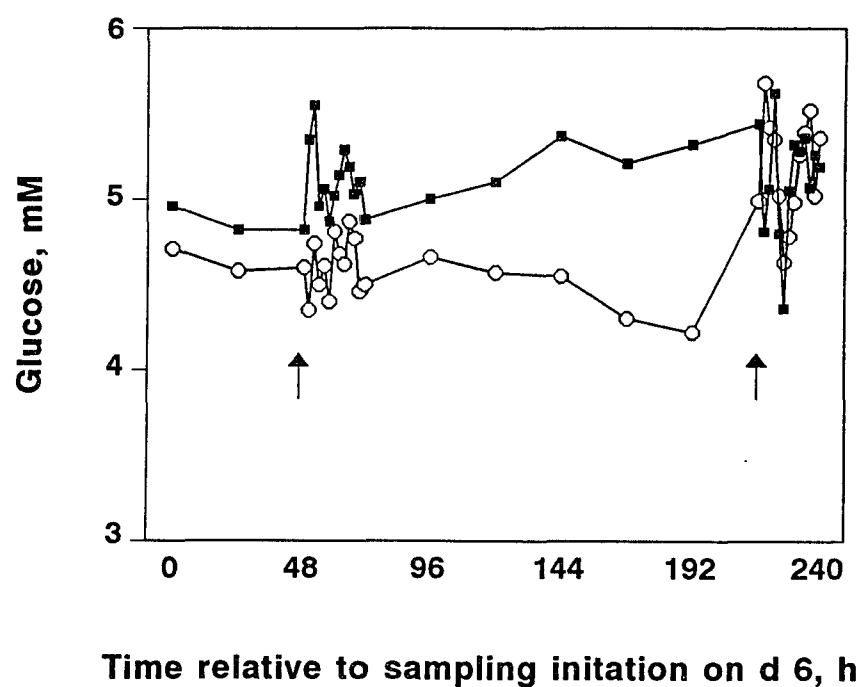


Figure 5.1. Plasma glucose concentrations during two dietary alterations. Mares received their first control treatment meal at 0800 on d 1. Mares were switched from 100 (control, ■) to 50% (restricted, ○) of their protein and energy requirements at 48 h (d 8, first arrow) and then switched from restricted to control at 216 h (d 15, second arrow).

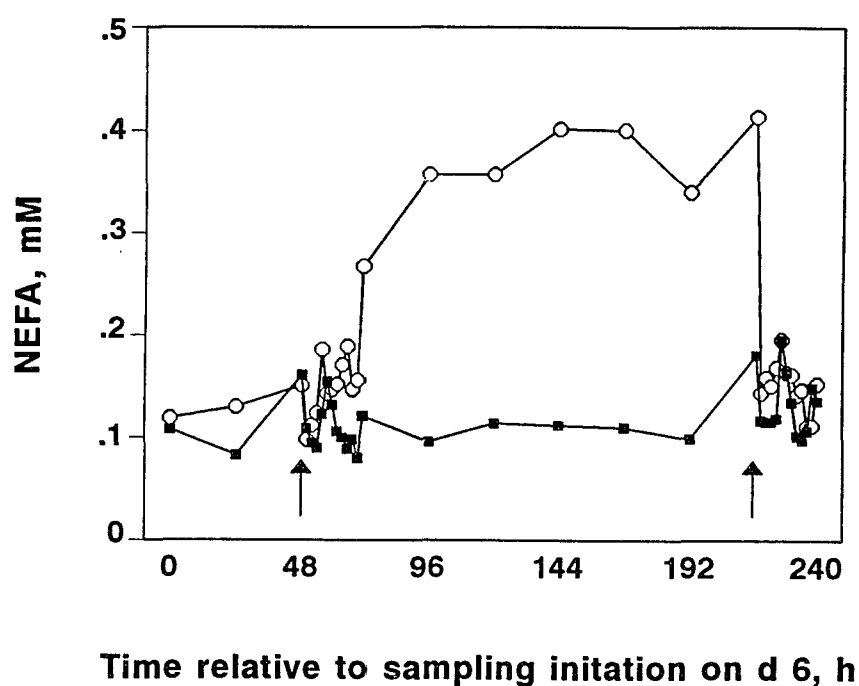


Figure 5.2. Plasma NEFA concentrations during two dietary alterations. Mares received their first control treatment meal at 0800 on d 1. Mares were switched from 100 (control, ■) to 50% (restricted, ○) of their protein and energy requirements at 48 h (d 8, first arrow) and then switched from restricted to control at 216 h (d 15, second arrow).

(time x diet interaction, $P = .009$). The elevated plasma NEFA concentrations in mares fed the restricted diet returned to control concentrations within 2 h following the first control meal, while plasma NEFA concentrations in mares fed the control diet remained similar throughout the 24 h period (time x diet interaction, $P = .04$).

All mares had similar ($P = .75$) plasma urea N concentrations before treatment initiation (Figure 5.3); however, mares fed the restricted diet had continually lowered (day x diet interaction, $P = .02$) concentrations during the daily sampling period. Plasma urea N concentrations were not altered within the first 24 h of dietary manipulation (time x diet interaction; control to restricted, $P = .21$, restricted to control, $P = .33$). However during both 24 h periods, feeding elevated plasma urea N concentrations (time effect; control to restricted, $P = .03$, restricted to control, $P = .009$).

Plasma Insulin and Glucagon Responses. Mean plasma insulin concentrations were similar ($P = .15$) when all mares were fed the control diet (Figure 5.4), then decreased in mares fed restricted protein and energy during the daily sampling period (diet effect, $P = .02$). The plasma insulin concentration response to feeding was diminished (time x diet interaction, $P = .09$) in mares which were switched to restricted protein and energy intakes; however, it was identical (time x diet interaction, $P = .36$) during refeeding of the control diet.

Mares switched to restricted intakes had similar plasma glucagon concentrations as mares fed the control diet during the first 24 h of dietary alteration (time x diet interaction, $P = .1$; Figure 5.5). Then during the period of

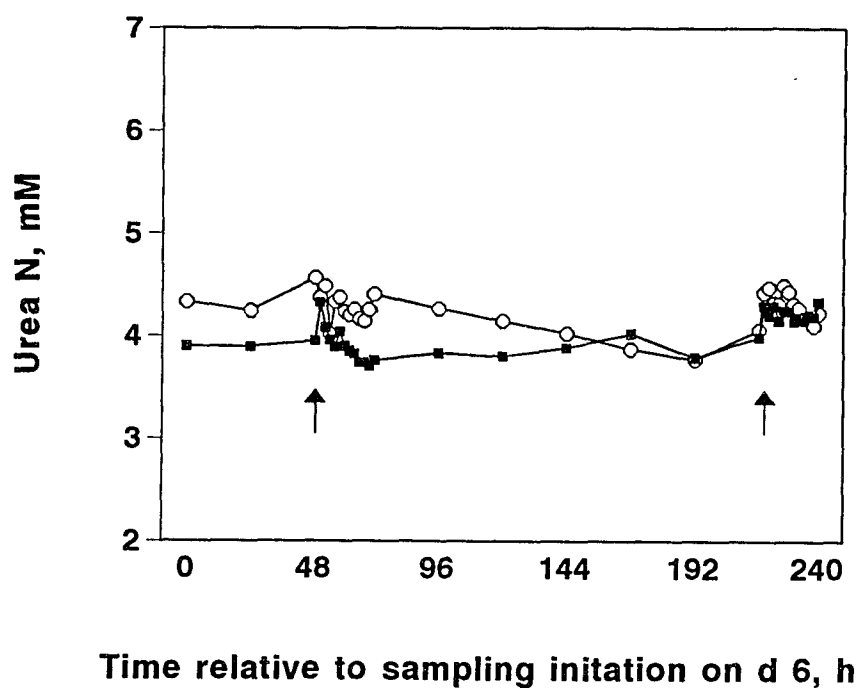


Figure 5.3. Plasma urea N concentrations during two dietary alteration. Mares received their first control treatment meal at 0800 on d 1. Mares were switched from 100 (control, ■) to 50% (restricted, ○) of their protein and energy requirements at 48 h (d 8, first arrow) and then switched from restricted to control at 216 h (d 15, second arrow).

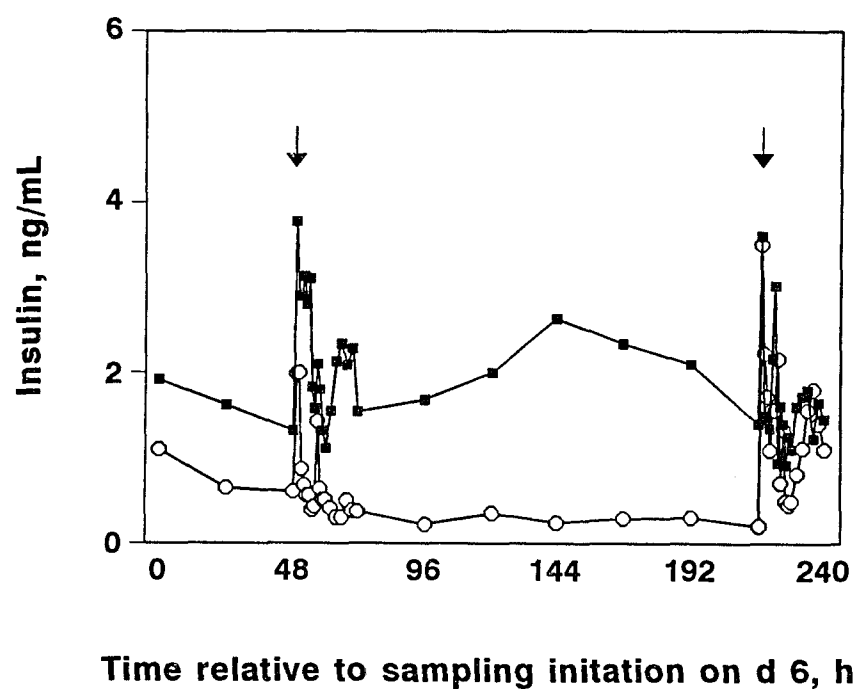


Figure 5.4. Plasma insulin concentrations during two dietary alteration. Mares received their first control treatment meal at 0800 on d 1. Mares were switched from 100 (control, ■) to 50% (restricted, ○) of their protein and energy requirements at 48 h (d 8, first arrow) and then switched from restricted to control at 216 h (d 15, second arrow).

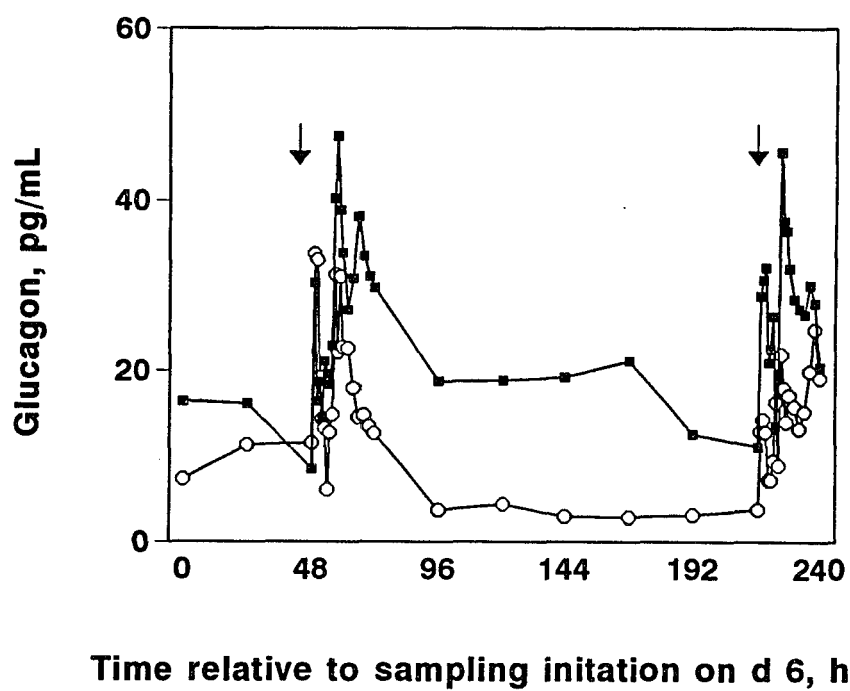


Figure 5.5. Plasma glucagon concentrations during two dietary alterations. Mares received their first control treatment meal at 0800 on d 1. Mares were switched from 100 (control, ■) to 50% (restricted, ○) of their protein and energy requirements at 48 h (d 8, first arrow) and then switched from restricted to control at 216 h (d 15, second arrow).

restricted intake, mean glucagon concentrations were reduced (diet effect, $P = .002$) in mares fed the restricted diet. When mares were refed the control diet, their plasma glucagon concentrations remained lower during the following 24 h (time x diet interaction, $P = .0007$). Feeding elevated plasma insulin (time effect, $P = .05$) and glucagon (time effect, $P = .0001$) concentrations during the 24 h following the change to the restricted diet, as well as during the refeeding of the control diet (time effect; $P = .0001$ and $.0001$, respectively).

Plasma IGF-1, Cortisol, and T_4 Responses. Plasma IGF-1 concentrations (Figure 5.6) changed similarly in all mares during the daily sampling period (day effect, $P = .002$) and did not vary with nutrient restriction (day x diet interaction, $P = .68$). Also, during both 24-h sampling periods, plasma IGF-1 concentrations were not influenced by dietary changes (time x diet interaction; control to restricted, $P = .34$, restricted to control, $P = .62$) or by feeding (time effect; control to restricted, $P = .4$, restricted to control, $P = .49$). Daily plasma cortisol concentrations were not influenced ($P = .14$) by restricted protein and energy intakes (Figure 5.7).

Protein and energy restriction did not influence plasma T_4 concentrations (Figure 5.8) during the daily sampling period (day x diet interaction, $P = .91$). During the first 24 h dietary alteration, mares which consumed the same diets had similar T_4 concentrations as those which switched diets (time x diet interaction; control to restricted, $P = .22$, restricted to control, $P = .7$). Nonetheless, mean plasma T_4 concentrations tended to be lower (diet effect, $P = .17$) in restricted

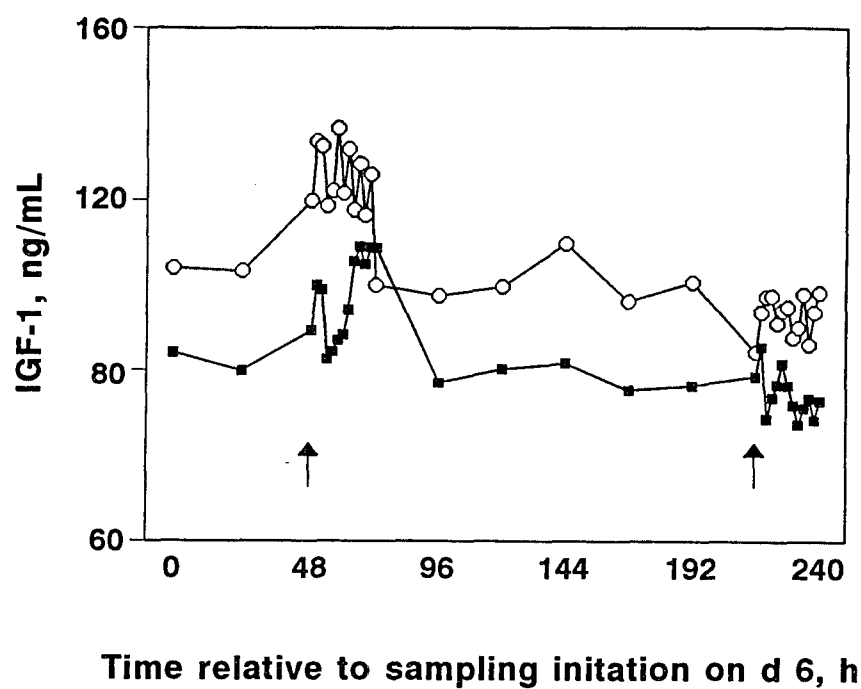


Figure 5.6. Plasma IGF-1 concentrations during two dietary alterations. Mares received their first control treatment meal at 0800 on d 1. Mares were switched from 100 (control, ■) to 50% (restricted, ○) of their protein and energy requirements at 48 h (d 8, first arrow) and then switched from restricted to control at 216 h (d 15, second arrow).

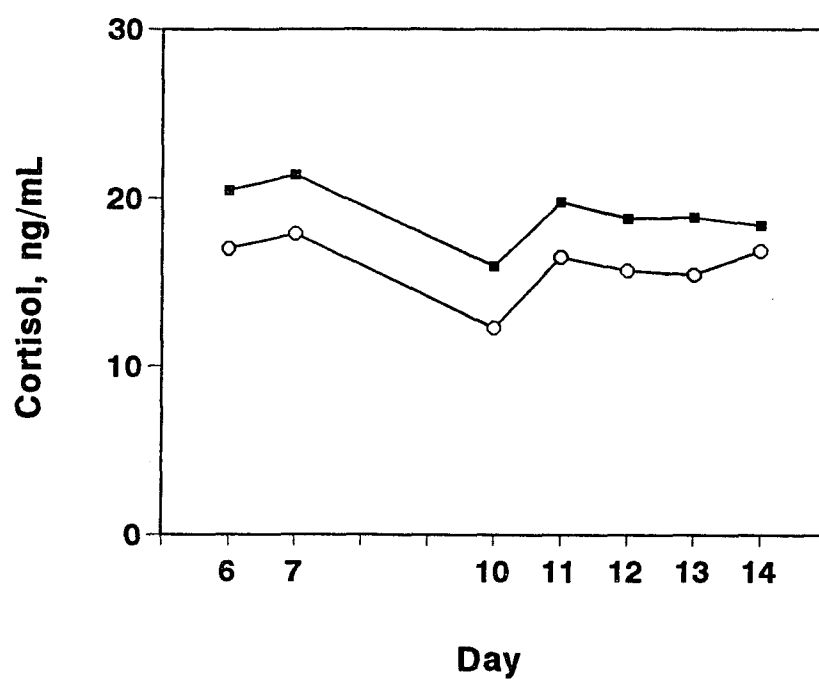


Figure 5.7. Plasma cortisol concentrations during two dietary alterations. Mares received their first control treatment meal at 0800 on d 1. Mares were switched from 100 (control, ■) to 50% (restricted, ○) of their protein and energy requirements on d 8.

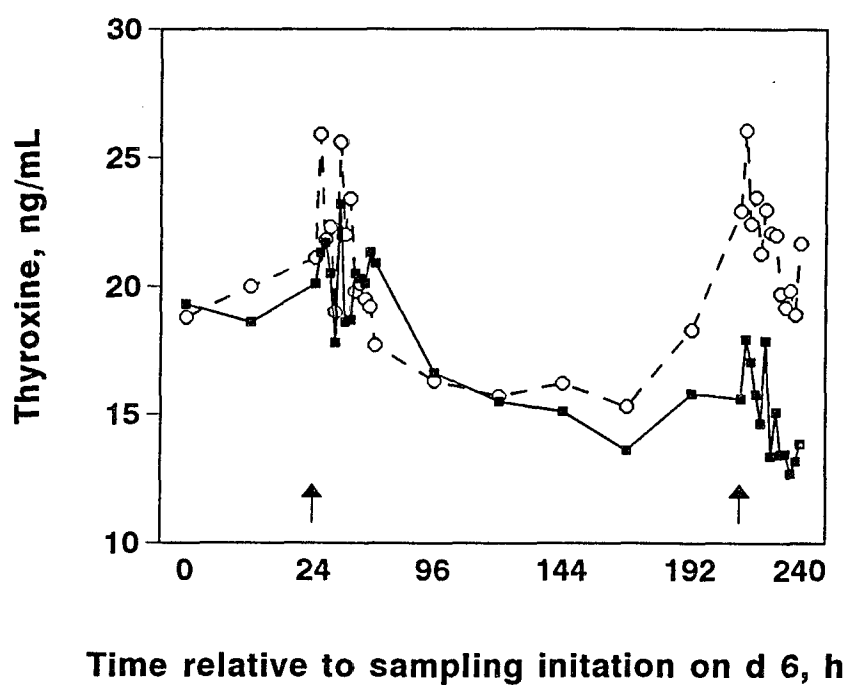


Figure 5.8. Plasma thyroxine concentrations during two dietary alterations. Mares received their first control treatment meal at 0800 on d 1. Mares were switched from 100 (control, ■) to 50% (restricted, ○) of their protein and energy requirements at 48 h (d 8, first arrow) and then switched from restricted to control at 216 h (d 15, second arrow).

mares during the 24-h period following refeeding of the control diet. Plasma T_4 concentrations increased after each meal during both 24-h sampling periods (time effect; control to restricted, $P = .04$, restricted to control, $P = .003$).

Discussion

This experiment confirms the rapid plasma increases of NEFA concentration during restriction of protein and energy intakes as observed in the first experiment (Chapter III) and describes even more rapid decreases of plasma NEFA concentrations during refeeding control intakes. Results from this experiment do not support the rapid alterations of plasma IGF-1 concentrations. The experiment also provides evidence of varied plasma metabolite and hormone responses in relation to feeding altered levels of protein and energy.

It appeared that the plasma glucose concentrations were lowered in response to feeding in mares switched to the restricted diet. These rapid alterations in response to feeding and the continued lower plasma glucose concentrations of those restricted mares were similar to reports by Blum et al. (1985) who observed rapid (within days) decreases in plasma glucose concentrations during restricted intakes (maintaining BW) in young steers, followed by rapid increases above control animals during refeeding. Rapid increased in plasma glucose concentrations were also observed after 3 d without feed in lambs (Cole et al., 1988) and 6 d without feed in lactating dairy cows (Reid et al., 1977). Similarly, in mares switched back to the control diet, the plasma glucose response to feeding was identical to control

mares. The varying responses of plasma glucose concentrations to control and restricted diets could conceivably be related to meal quality as shown by Stull and Rodiek (1988); a meal consisting of alfalfa/corn or corn to meet 25% of the daily DE requirements for adult horses elevated plasma glucose and insulin concentrations to a greater extent than an isocaloric meal of alfalfa or corn/corn oil. However, in peripubertal gilts, rises in plasma glucose concentrations were similar after feeding regardless of intake (Prunier et al., 1993).

In ruminants, plasma NEFA concentrations increase slowly (within days) during feed deprivation and return to normal concentrations immediately (before 1 d) with realimation (Pothoven and Beitz, 1975; Reid et al., 1977; Cole et al., 1988). Comparable results are observed in steers during reduced intakes and realimation (Blum et al., 1985; Ellenberger et al., 1989). Likewise, in the mares that were switched to restricted intakes, plasma NEFA concentrations began to rise after the initial meal and continued to rise several hours following the second meal, peaking 2 d after the alteration. However, plasma NEFA concentrations returned to control levels by 2 h after the first control meal on d 15. It appears that lipogenesis and(or) lipolysis responded faster to realimation than to restriction in the horse, as was also shown in steers (Pothoven and Beitz, 1975).

Cole et al. (1988) reported elevated plasma urea N concentrations in lambs during a 72 h period without feed, with concentrations remaining elevated through the first feeding and returning to normal by the second day of feeding. However, in these mares, plasma urea N concentrations slowly continued to decrease during

protein and energy restriction. This was not observed in the first experiment (Chapter II); mares fed both the control and restricted protein and energy diets had similar plasma urea N concentrations throughout the first experiment. Blum et al. (1985) and Ellenberger et al. (1989) reported a slow decline in plasma urea N concentrations during realimation following restricted intakes in steers. However, during the first 24 h of realimation in these mares, plasma urea N appeared uninfluenced by dietary intake. The equal protein:energy ratios in these diets likely explains the similar plasma urea N concentrations. The ratio between protein and energy intake appears to most influence plasma urea N concentrations (Hammond, 1983), whereas low protein intakes alone reduce plasma urea N (Preston et al., 1965; Eggum, 1970) and low energy intakes alone increase plasma urea N concentrations (Hammond, 1983). Similar to the results of the first experiment (Chapter II) and to reports in ruminants (Manston et al., 1981; Hammond, 1983; McCarthy et al., 1992) and horses (DePew et al., 1994), small but significant meal-induced increases in plasma urea N concentrations were observed.

Cole et al. (1988) reported that plasma insulin concentrations in lambs, which were reduced during 72 h of feed deprivation, were elevated above those in control lambs before the second day of realimation. In response to feed restriction and realimation, immediate alterations (within days) in plasma insulin concentrations were also observed in steers by Blum et al. (1985). In the present experiment, a reduction in meal-induced plasma insulin concentrations was observed in mares following their first restricted meal; however, immediately after the refeeding of the

first control meal, plasma insulin concentrations mirrored that of mares which were fed the control diet during the entire experiment. Prefeeding responses of plasma insulin concentrations to dietary alterations might not fully explain metabolic consequences of nutrient restriction. Others have observed no influences of restriction on prefeeding plasma insulin concentrations in horses (Glade et al., 1984) and gilts (Prunier et al., 1993).

Plasma glucagon concentrations reported in these mares before feeding, also observed in horses by Lucke and Hall (1980), were low compared with ruminants (Bassett, 1972; Sahlu et al., 1992). Restriction of protein and energy diminished plasma glucagon concentrations before and in response to feeding, but not within the first day of dietary restriction. Others observed no changes in plasma glucagon concentrations during protein restriction in goats (Gaskins et al., 1991; Sahlu et al., 1992). Both morning and evening meals increased plasma glucagon concentrations in these mares, as was also observed in mares and stallions (DePew et al., 1994), sheep (Bassett, 1972), and goats (de Jong, 1981b). Increased circulating amino acids, previously observed after feeding in the horse (Johnson and Hart, 1974; DePew et al., 1994), stimulate glucagon secretion (Kuhara et al., 1991) for the rapid conversion of amino acids to glucose (Guyton, 1991). However, glucagon did not change in relation to feeding in goats (de Jong, 1981a), or in cows during the dry period, in a feed restriction induced ketonemia, or during a recovery period (deBoer et al., 1985).

In ruminants, long term nutritional deficiencies reduce plasma IGF-1 concentrations (Houseknecht et al., 1988; Elsasser et al., 1989; Granger et al., 1989). However, short term alterations in plasma IGF-1 concentrations have also been observed. Buonomo and Baile (1991) reported elevated plasma IGF-1 concentrations within 4 h after feeding barrows that were previously held without feed for 48 h. Clemmons and Underwood (1991) also reported steady increases, of plasma IGF-1 concentrations in humans beginning immediately following refeeding after 4 d without feed. The immediate alterations in plasma IGF-1 concentrations that were noted in the first experiment (Chapter II) were not observed in this experiment. Also, no meal induced alterations in plasma IGF-1 concentrations were observed in these mares.

Glade et al. (1984) reported higher plasma cortisol concentrations in weanlings fed 160 vs 80% of their protein and energy requirements (60.8 vs 48.7 ng/mL, respectively). However in this experiment, protein and energy restriction did not alter plasma cortisol concentrations. Because of the metabolic role of cortisol in increasing gluconeogenesis, lipolysis, and protein catabolism (Guyton, 1991), it would seem that nutrient restriction would increase circulating cortisol concentrations to facilitate nutrient mobilization; however, varying results are observed. Indeed, some researchers have observed increased plasma cortisol concentrations during hypoglycemia in horses (James et al., 1970) and 5 h after feeding peripubertal gilts a diet which was 50% ad libitum intake (Pruiner et al. 1993). In contrast, Schrick et al. (1990) reported similar daily peripheral plasma

cortisol concentration in cows (100 d postcalving) fed either at high or low energy intakes. And still others have reported higher plasma cortisol concentrations in lambs fed high compared to moderate protein levels at two environmental temperatures (Ghani, 1988).

Because of the role of thyroid hormones in increasing gastrointestinal tract secretion and motility and their role in carbohydrate (increased glucose absorption and tissue uptake) and fat metabolism (Guyton, 1991), increases in plasma thyroid hormone concentrations following feeding would appear consistent with its actions. Indeed, not only were plasma T_4 concentrations elevated after meals in the present experiment, but others also have also reported meal induced increases in plasma T_4 concentrations in horses (Glade et al., 1984; Youket et al., 1985) and in pigs (Buonomo and Baile, 1991). However, as observed in the present experiment, altered dietary protein and energy intakes did not influence the feeding responses of plasma T_4 concentrations in horses (Glade and Reimers, 1985). Evidence supporting the unaltered plasma thyroid hormone concentrations during dietary protein and energy restriction includes results from the first experiment (Chapter II), and Glade et al. (1984), and Glade and Reimers (1985). However, rapid reductions in circulating thyroid hormone concentrations were reported with reduced feed intake in steers (Blum et al., 1985; Ellenberger et al., 1989) and rats (Schalch and Cree, 1985).

In summary, rapid alterations were observed for plasma glucose, NEFA, and insulin concentrations, whereas no responses of plasma urea N, IGF-1, cortisol, or

T_4 concentrations occurred during dietary alterations. During the first feeding period, when mares were switched to restricted protein and energy intakes, the plasma glucose and insulin response was reduced, while the plasma NEFA response was elevated. Subsequently, when mares were returned to control diets, plasma glucose and insulin were elevated, while NEFA concentrations returned immediately to normal. Meal-induced increases in plasma glucose, insulin, urea N, glucagon and T_4 concentrations were observed.

Implications

It appears that several plasma metabolites and hormones can be altered immediately after dietary modifications. This experiment provides evidence of the importance of early sampling when monitoring plasma constituents during nutrient alterations and also suggests that prefeeding responses of plasma metabolite and hormonal concentrations alone will not fully explain the metabolic consequences of nutrient restriction. The results also stress the importance of sampling prior to changes in dietary regimens to provide appropriate background information.

CHAPTER VI

FEED DEPRIVATION IN MARES: CHARACTERIZATION OF PLASMA
METABOLIC AND HORMONAL CONCENTRATIONS AND
RESPONSES TO EXERCISE

Introduction

Plasma lipid responses in horses or ponies to feed deprivation or disease have been demonstrated (Naylor et al, 1980; Morris et al., 1972). However, appropriate reference of the response of β -hydroxybutyrate, urea N, glucagon, prolactin, thyroid hormones, IGF-1, and GH to feed deprivation in horses has not been reported. In horses, feed deprivation has been shown to increase plasma lactate (Baetz and Pearson, 1972; Rose and Sampson, 1982), urea N (Patterson et al., 1985), and β -hydroxybutyrate concentrations (Rose and Sampson, 1982), to decrease plasma prolactin (DePew et al., 1994) and urea N concentrations (Baetz and Pearson, 1972), to not influence plasma glucose (Baetz and Pearson, 1972; Freestone et al., 1991), insulin, and cortisol concentrations (Freestone et al., 1991). Decreased plasma IGF-1 concentrations during feed deprivation were reported in humans (Merimee et al., 1982), swine (Buonomo and Baile, 1991) and bulls and steers (Ward et al., 1992). Others have reported reduced T_3 and T_4 concentrations in feed-deprived sheep (Blum et al., 1980), cattle (Blum et al., 1981; Blum and Kunz, 1981; Tveit and Larsen, 1983), and pigs (Buonomo and Baile, 1991). Feed deprivation increased plasma GH concentrations in men (Ho et al., 1988), swine

(Buonomo and Baile, 1991), and sheep (Driver and Forbes, 1981; Cole et al., 1988); decreased plasma GH concentrations in steers (Rule et al., 1985); and did not alter plasma GH concentrations in calves and sheep (McAttee and Trenkle, 1971a; Trenkle, 1976). The objectives of this experiment were to characterize plasma NEFA, β -hydroxybutyrate, lactate, glucose, urea N, insulin, glucagon, prolactin, IGF-1, cortisol, T_3 , T_4 , and GH concentrations and the responses to exercise during feed deprivation in mares.

Experimental Procedures

Animals and Dietary Treatments. Twelve light horse mares (8 to 9 yr of age, 402 to 462 kg initial BW) grazing native grass pasture were stratified by weight (6 horses/treatment) in a completely randomized design to one of two dietary treatments. The two treatments included either fed mares (control; fed 100% of the protein and energy requirement for maintenance; NRC, 1989) or unfed mares (deprived). The experiment was conducted during October, 1993. Horses were offered Alicia bermuda grass hay at .6 kg of DM/100 kg of BW, which provided 30% of the CP requirement from the hay in the control diet. The bermuda grass hay (DM basis) analyzed 6.5% CP, 45.2% ADF, 65.0% NDF and was calculated to contain 1.78 Mcal DE/kg (NRC, 1982). A control supplement was formulated and added at .6 kg of DM/100 kg of BW to provide the appropriate amount of CP and DE (Table 3.1). The control diet met the requirements for Ca, P, Mg, Na, K, and S. Before initiation of the experiment, horses were adapted in the pasture to the

hay for 10 d and to the control supplement for 5 d. All mares consumed the control diet from d 1 to d 7. On d 8, feed was removed from half of the mares (deprived mares) 2 h after the morning meal, while the remaining mares (control mares) consumed the control diet during the entire experiment. Horses were housed in stalls during the entire study, offered equal portions of their ration at 0800 and 1600, and allowed continual access to water.

Weighing and Blood Sampling, Processing, and Analysis. Mares were weighed at 0700 (15 h after the last afternoon feeding) on d 8 (before feed deprivation) and on d 11 (69 h after feed removal). Blood (7 mL) was obtained at 0700 and 2000 (1 h prior to both feedings) on d 8, 9, and 10, and at 0700 on d 6, 7, and 11 by jugular venipuncture into two evacuated tubes, one containing potassium oxalate and sodium fluoride, and the other containing K₃EDTA (Sherwood Medical, St. Louis, MO). Day 9, 10, and 11 represent 21, 45, and 69 h of feed deprivation, respectively. Two mL of blood from the sodium fluoride tube was added to 2 mL of 1 N perchloric acid for β -hydroxybutyrate analysis and 3 mL of blood from the K₃EDTA was added to 37.5 μ L of benzamidine HCl (protease inhibitor, 2.401 M solution) for glucagon and insulin analysis.

On d 10 at 0600, horses were fitted with 14-ga indwelling jugular catheters. Blood (3 mL) was drawn from 0700 (45 h after feed removal, 1 h before morning feeding) to 1600 (prior to afternoon feeding) at 15 min intervals for characterization of GH. Blood was placed into 12 x 75 mm glass tubes containing 100 USP units of lithium heparin in 100 μ L of saline. During bleeding periods, control mares were

given 3 h to consume the morning meal (supplement given first, 30 min prior to hay), and any remaining feed was added to the afternoon meal. All mares were allowed free access to water during the bleeding periods. On d 11 at 1100 (73 h after feed withdrawal), mares were exercised by a 5 min longe at the trot, 3 h after the morning meal. Blood (7 mL) was drawn every 10 min from 20 min before to 90 min after initiation of the exercise and was placed into evacuated tubes containing potassium oxalate and sodium fluoride.

Plasma was analyzed for glucose, NEFA, Urea N, IGF-1, Cortisol, T₃, T₄, insulin, glucagon, prolactin, lactate, and β -hydroxybutyrate (daily samples), and GH, prolactin, glucose, NEFA, lactate, and cortisol (exercise).

Blood Processing and Analyses. Blood samples were placed in a 5°C refrigerator immediately after withdrawal and were centrifuged within 1 h at 1500 x g for 15 min. Plasma was harvested and stored at -15°C. Commercial kits were used in the colorimetric determinations of plasma glucose (Sigma Tech. Bull. No. 315, Sigma Chemical), and NEFA (NEFA-C kit, Wako Chemicals USA, Richmond, VA). Plasma urea N, insulin, glucagon was analyzed as described by DePew et al. (1994). Cortisol, IGF-1, prolactin, and GH were analyzed as previously described (Thompson et al., 1988; Granger et al., 1989; Colborn et al., 1991a; Thompson et al., 1992, respectively) and β -hydroxybutyrate was analyzed by the procedure of Williamson and Mellanby (1965). Dietary CP was determined by the Kjeldahl procedure (AOAC, 1984) and NDF and ADF were by the method of Goering and VanSoest (1970).

Calculations and Statistical Procedures. Net areas under the response curve to exercise were computed by summing the time x concentration increments for each mare after subtraction of that animal's pretreatment average. Basal concentrations were computed as the average of the samples taken immediately prior to the initiation of exercise or feed deprivation. Where basal concentrations were different between treatments, the unit increase over basal concentrations were reported and statistically analyzed. The GH data collected on d 10 were analyzed via PULSAR (Gitzen and Ramirez, 1976), with parameters set as described by Thompson et al. (1992). Analyses of daily plasma metabolite and hormonal concentrations only included samples taken during the treatment period. Statistical analyses were conducted using the GLM procedure of SAS (1988). Included in the completely randomized design model was dietary treatment, which was tested with the residual error. With samples taken over time, multivariate repeated measures analysis included testing time and time x diet. Where appropriate, the LSD test was used to separate treatment differences at specific time points. Wilks' Lambda probability was reported; when an insufficient degrees of freedom occurred (number of repeated measures exceeded error degrees of freedom), the multivariate Greenhouse-Geisser adjusted probability was used (SAS, 1988).

Results

Body Weight Change and GH Response. During the period of feed withdrawal, mares lost 3.72 kg/d, while mares consuming feed gained .72 kg/d ($P =$

.0001; SE = .45). Plasma GH characteristics were not altered by 45 h of feed deprivation (Table 6.1). All detected peaks occurred approximately 2 to 5 h after the morning meal regardless of whether the mares were eating.

Daily Plasma Concentrations. Plasma NEFA concentrations were similar ($P = .52$) in all mares prior to, but increased within 21 to 45 h after, feed withdrawal (day x diet interaction, $P = .01$; Figure 6.1). Likewise, feed deprivation increased the initially similar ($P = .34$) plasma β -hydroxybutyrate concentrations within 21 to 45 h (day x diet interaction, $P = .002$; Figure 6.1).

Plasma lactate concentrations were increased during feed deprivation (day x diet interaction, $P = .02$; Figure 6.2); however, feed deprivation did not influence plasma glucose concentrations during the experiment (day x diet interaction, $P = .71$; Figure 6.2). Mares had similar ($P = .76$) plasma urea N concentrations before feed withdrawal and then exhibited elevated plasma urea N concentrations during the period of feed deprivation (day x diet interaction, $P = .02$; Figure 6.2).

Similar initial plasma insulin concentrations ($P = .45$) before feed removal remained unchanged during feed deprivation (day x diet interaction, $P = .66$; Figure 6.3). Withdrawal of feed increased the initially similar ($P = .65$) plasma glucagon concentrations within 45 h (day x diet interaction, $P = .06$; Figure 6.3).

Plasma prolactin concentrations were not influenced by feed restriction (day x diet interaction, $P = .25$); however, they varied between sampling times (day effect, $P = .002$; Figure 6.4). Feed deprivation reduced plasma IGF-1 concentrations (day x diet interaction, $P = .02$; Figure 6.4) which were similar

Table 6.1. Growth hormone secretion measurements relative to two meals.
 Adult mares were fed 100 (control) or 0% (deprived for 45 h)
 of protein and energy requirement

Item	Control	Deprived	SE
n	6	6	
Baseline GH concentration, ng/mL	.78	.76	.06
Average GH concentration, ng/mL	.97	1.1	.13
Number of GH peaks	2.0	1.7	.39
GH peak amplitude, ng/mL	21.0	27.6	9.03

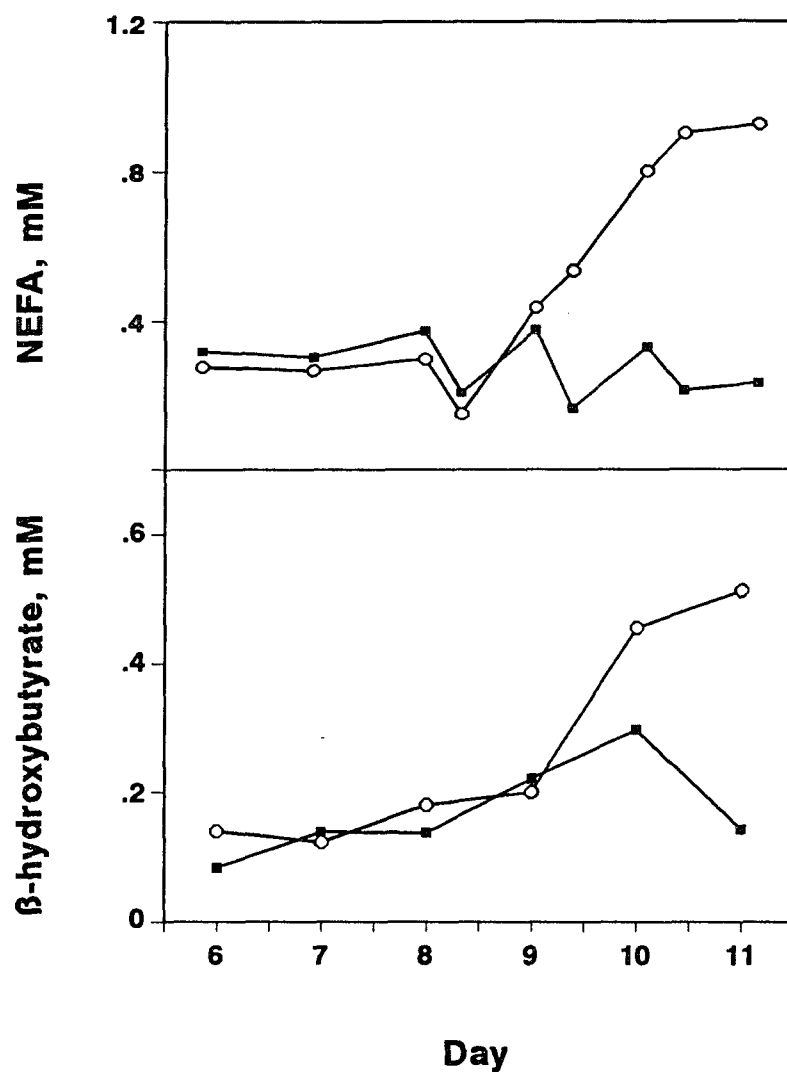


Figure 6.1. Daily concentrations of plasma NEFA and β -hydroxybutyrate. Adult mares were fed 100 (control; ■) or 0% (deprived; ○) of protein and energy requirement. Mares received their first control meal at 0800 on d 1. Feed was removed from deprived mares at 1000 on d 8 (2 h after their morning meal). Day 9, 10, and 11 represents 21, 45, and 69 h after feed removal. SE were .04 and 33.6 mM for NEFA and β -hydroxybutyrate, respectively.

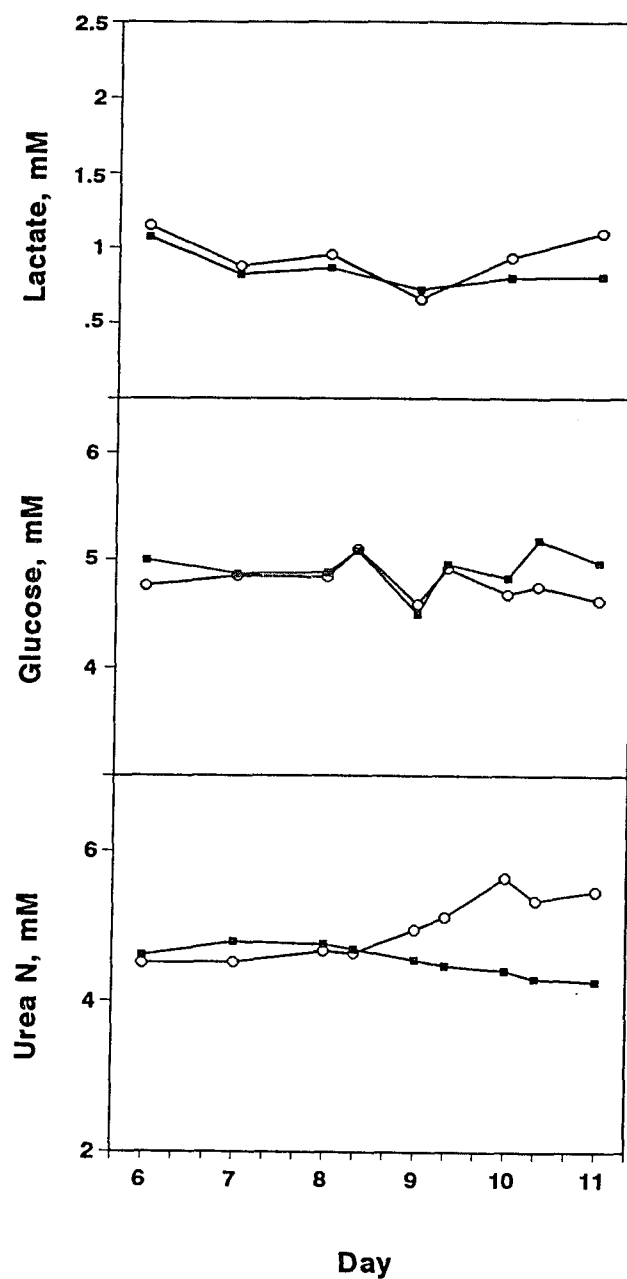


Figure 6.2. Daily concentrations of plasma lactate, glucose, and urea N. Adult mares were fed 100 (control; ■) or 0% (deprived; ○) of protein and energy requirement. Mares received their first control meal at 0800 on d 1. Feed was removed from deprived mares at 1000 on d 8 (2 h after their morning meal). Day 9, 10, and 11 represents 21, 45, and 69 h after feed removal. SE were .11, .18, and .12 mM for lactate, glucose, and urea N, respectively.

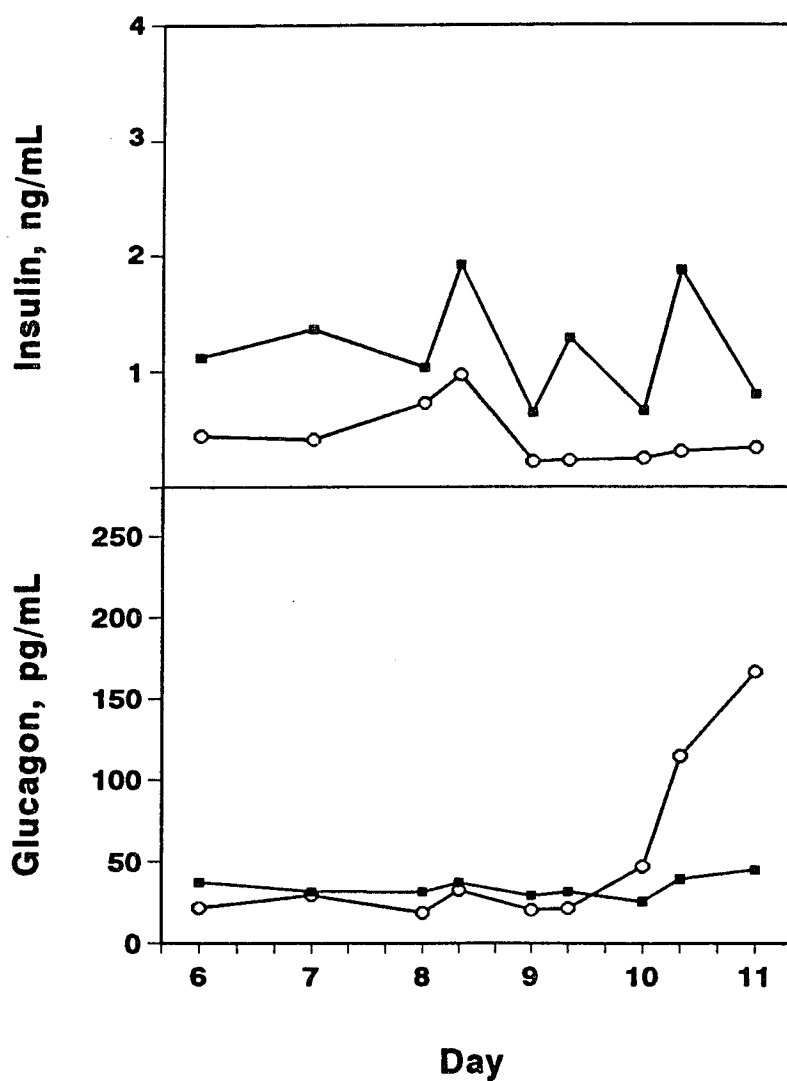


Figure 6.3. Daily concentrations of plasma insulin and glucagon. Adult mares were fed 100 (control; ■) or 0% (deprived; ○) of protein and energy requirement. Mares received their first control meal at 0800 on d 1. Feed was removed from deprived mares at 1000 on d 8 (2 h after their morning meal). Day 9, 10, and 11 represents 21, 45, and 69 h after feed removal. SE were .27 ng/mL and 16.3 pg/mL for insulin and glucagon, respectively.

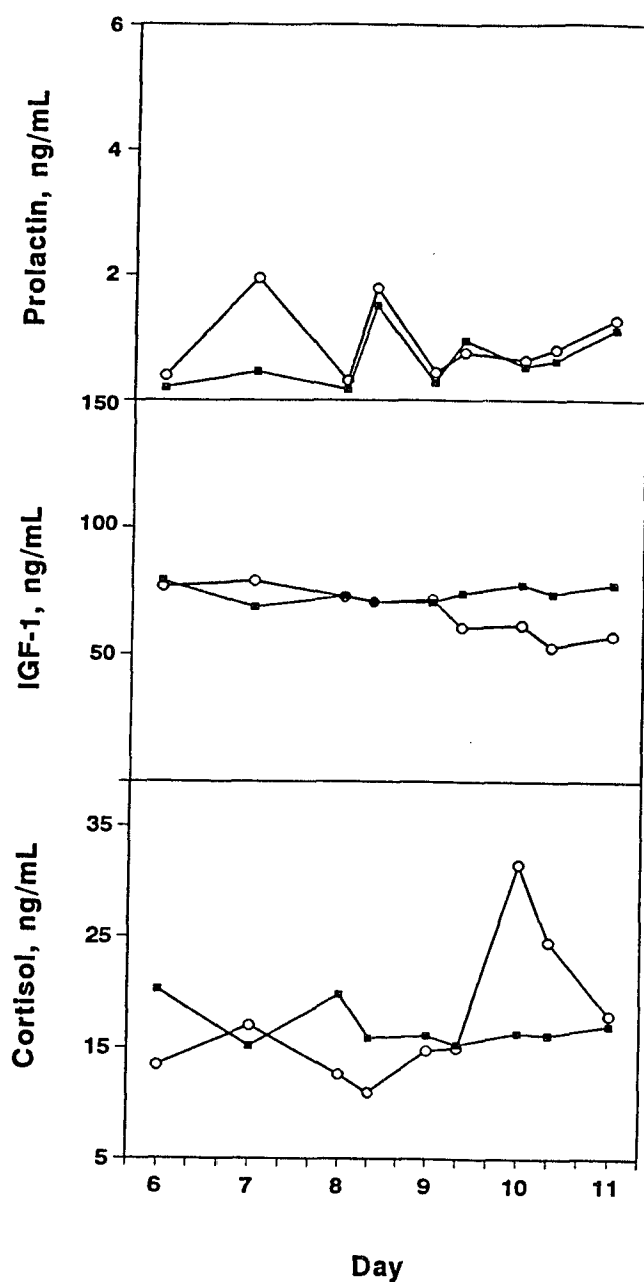


Figure 6.4. Daily concentrations of plasma prolactin, IGF-1, and cortisol. Adult mares were fed 100 (control; ■) or 0% (deprived; ○) of protein and energy requirement. Mares received their first control meal at 0800 on d 1. Feed was removed from deprived mares at 1000 on d 8 (2 h after their morning meal). Day 9, 10, and 11 represents 21, 45, and 69 h after feed removal. SE were .46, 4.1, and 2.6 ng/mL for prolactin, IGF-1, and cortisol, respectively.

($P = .77$) before feed removal. Plasma cortisol concentrations were similar ($P = .28$) in all mares before feed withdrawal (Figure 6.4), and were not influenced by feed deprivation (day x diet interaction, $P = .2$). There were no influences of feed deprivation on thyroid hormone concentrations (day x diet interaction; T_3 , $P = .51$; T_4 , $P = .52$; Figure 6.5).

Responses to Exercise. All plasma constituents taken prior to exercise varied between dietary treatments ($P < .05$; Table 6.2), therefore data reported in figures are residuals relative to each horses basal concentrations. Plasma NEFA concentrations were increased (time effect, $P = .0006$) 20 min after initiation of exercise and remained elevated in fed mares, but were rapidly reduced in mares which were feed deprived (time x diet interaction, $P = .006$; Figure 6.6). No dietary induced alterations in plasma lactate concentration to exercise were observed (time x diet interaction, $P = .83$; Figure 6.6). Feed deprivation elevated, whereas feed consumption reduced, plasma glucose concentrations following exercise (time x diet interaction, $P = .07$; Figure 6.6).

Exercise elevated (time effect, $P = .03$) plasma prolactin concentrations by 10 min after initiation of exercise (Figure 6.7). Plasma prolactin concentrations of feed-deprived mares remained elevated throughout the sampling period, but returned to below baseline concentrations in fed mares (time x diet interaction, $P = .09$). Plasma cortisol concentrations were not influenced by the exercise (time effect = .58) or feed deprivation during exercise (time x diet interaction, $P = .4$; Figure 6.7). Exercise elevated (time effect, $P = .003$) plasma GH concentration by

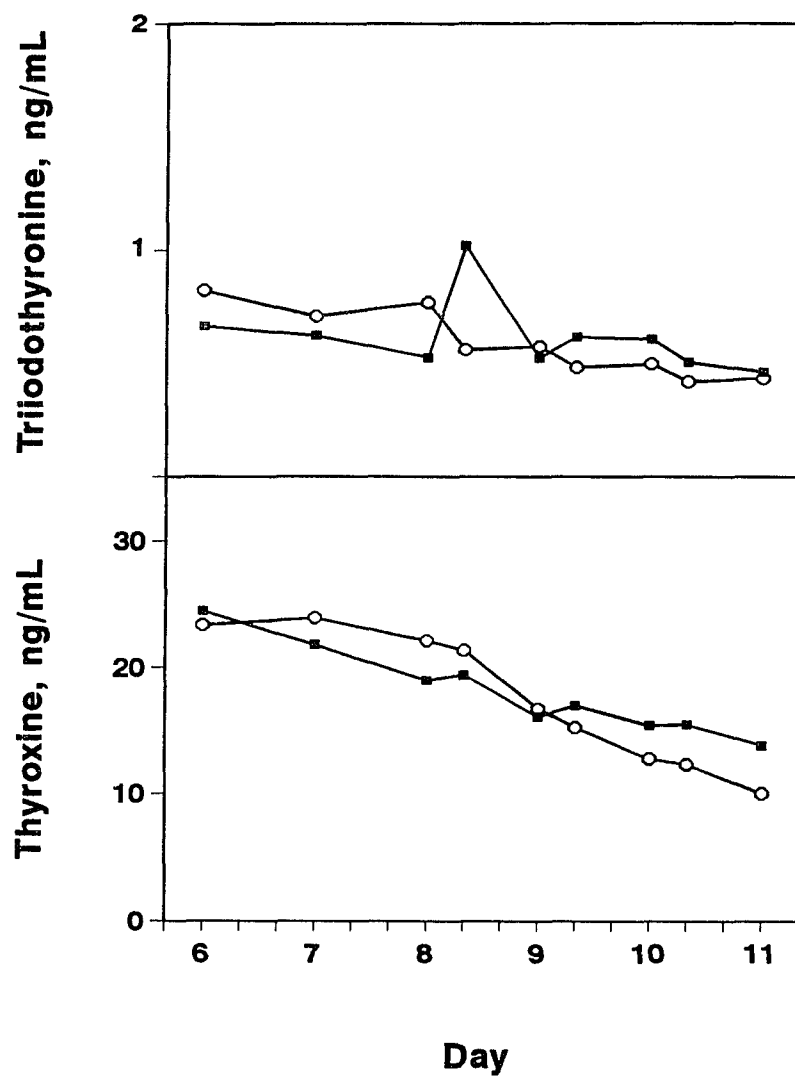


Figure 6.5. Daily concentrations of plasma triiodothyronine and thyroxine. Adult mares were fed 100 (control; ■) or 0% (deprived; ○) of protein and energy requirement. Mares received their first control meal at 0800 on d 1. Feed was removed from deprived mares at 1000 on d 8 (2 h after their morning meal). Day 9, 10, and 11 represents 21, 45, and 69 h after feed removal. SE were .11 and 1.7 ng/mL for triiodothyronine and thyroxine, respectively.

Table 6.2. Kinetic parameters relative to exercise. Adult mares were fed 100 (control) or 0% (deprived for 73 h) of protein and energy requirements

Item	Control	Deprived	SE	P-value
n	6	6		
Nonesterified fatty acids				
Basal concentrations, mM	.11	.93	.06	.0001
Curve area, min • mM	6.15	2.04	2.2	.21
Lactate				
Basal concentrations, mM	.88	1.28	.06	.001
Curve area, min • mM	17.9	23.4	10.1	.72
Glucose				
Basal concentrations, mM	5.65	4.67	.24	.016
Curve area, min • mM	-33.8	21.5	12.5	.01
Prolactin				
Basal concentrations, ng/mL	1.42	.31	.25	.01
Curve area, min • ng/mL	115.9	144.2	45.3	.67
Cortisol				
Basal concentrations, ng/mL	15.1	24.3	2.35	.019
Curve area, min • ng/mL	201.7	84.1	206.8	.7
Growth hormone				
Basal concentrations, ng/mL	.83	1.44	.16	.02
Curve area, min • ng/mL	142.7	286.9	90.6	.29

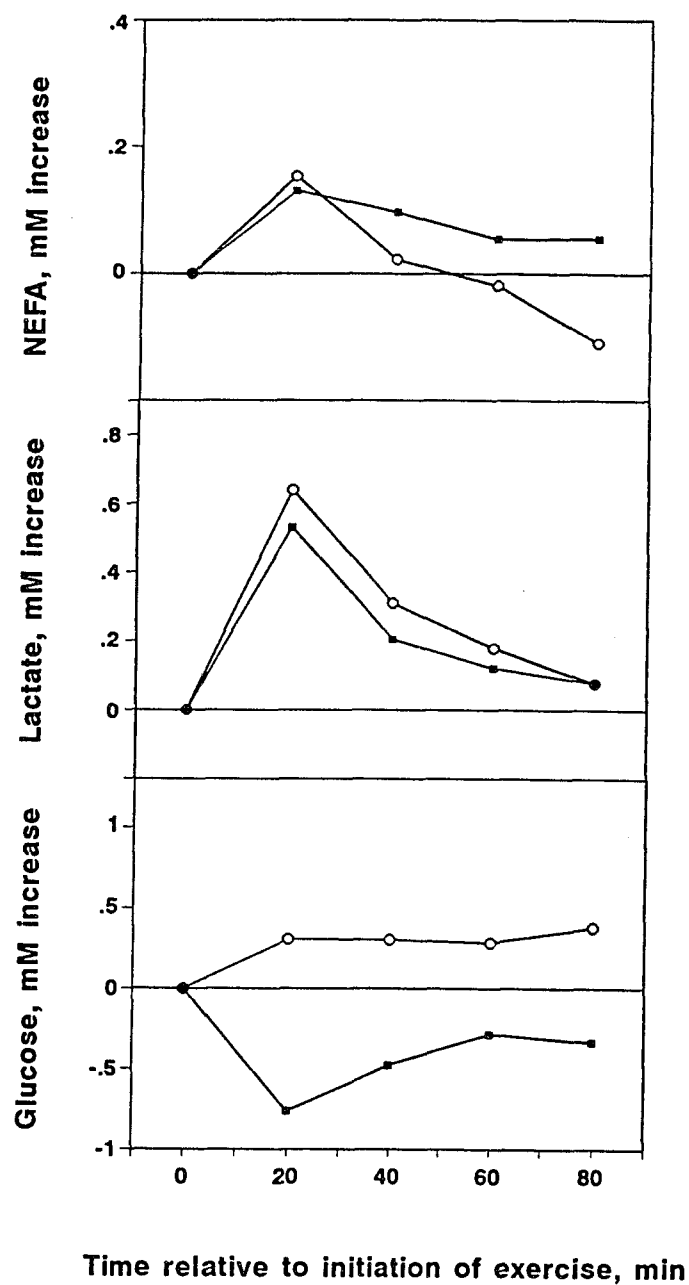


Figure 6.6. Increase above basal concentrations of plasma NEFA, lactate, and glucose relative to a 5 min exercise bout. Adult mares were fed 100 (control; ■) or 0% (deprived; ○) of protein and energy requirement. Feed was removed from deprived mares 73 h before initiation of exercise. Basal concentrations are reported in Table 6.3. SE were .02, .12, and .11 mM for NEFA, lactate, and glucose, respectively.

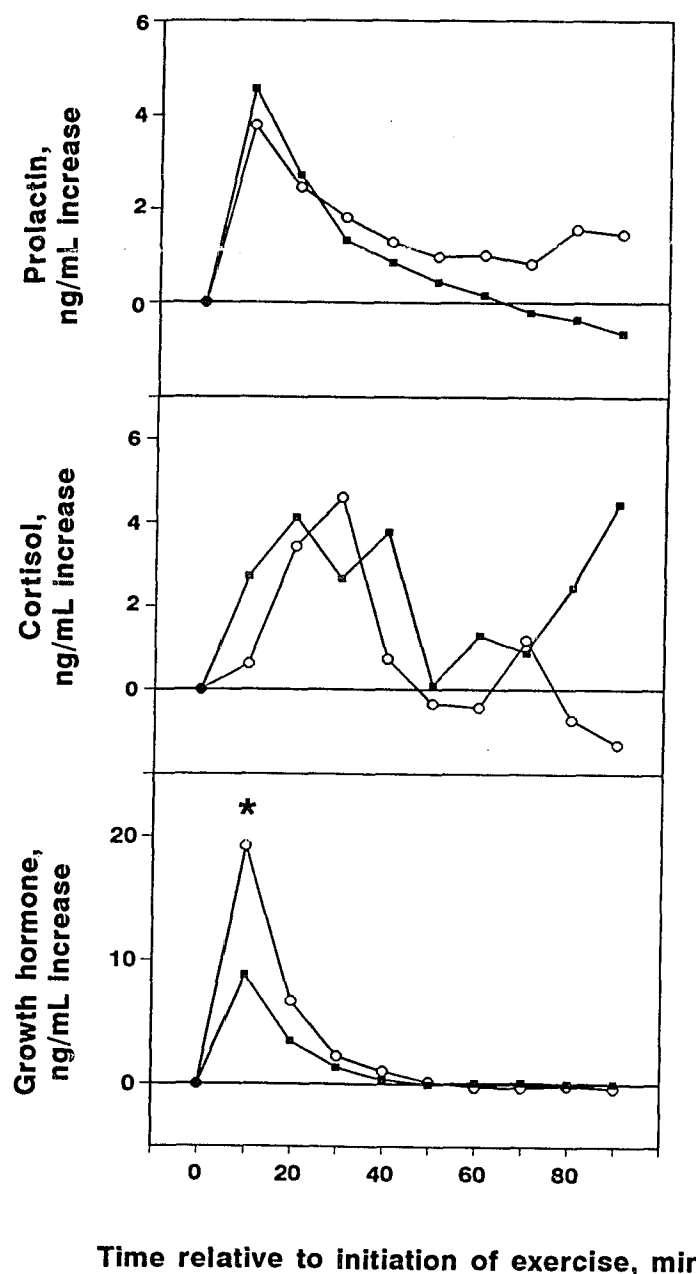


Figure 6.7. Increase above basal concentrations of plasma prolactin, cortisol, and growth hormone relative to a 5 min exercise bout. Adult mares were fed 100 (control; ■) or 0% (deprived; ○) of protein and energy requirement. Feed was removed from deprived mares 73 h before initiation of exercise. Basal concentrations are reported in Table 6.3. The astrisk indicates a difference between control and deprived mares at that time point ($P = .0001$). SE were .54, 1.6, and 1.8 ng/mL for prolactin, cortisol, and growth hormone, respectively.

10 min after initiation of exercise (Figure 6.7). Feed deprived mares had greater ($P = .0001$) plasma GH concentrations only at 10 min after initiation of exercise.

Discussion

Increases in plasma NEFA concentrations within 21 h of feed deprivation in these mares were consistent with earlier reports in horses (Baetz and Pearson, 1972; Naylor et al., 1980; Rose and Sampson, 1982). Increased lipolysis and(or) decreased lipogenesis, related to changes in either circulating insulin, glucagon, epinephrine, norepinephrine (Rose and Sampson, 1982), GH, thyroid stimulating hormone, or ACTH (Guyton, 1991) elevate plasma NEFA concentrations. Similar responses of plasma NEFA concentrations to feed deprivation was reported in swine (Baetz and Mengeling, 1971), sheep (Heitmann et al., 1986), cattle (Rule et al., 1985; Ward et al., 1992), and humans (Ho et al., 1988).

In the horse, plasma NEFA concentrations increase during exercise or recovery (Snow and Mackenzie, 1977; Deldar et al., 1982; Rose and Sampson, 1982; Miller-Graber et al., 1991; Lawrence et al., 1993). Similarly, in the present experiment, elevated plasma NEFA concentrations were observed following exercise and remained elevated in fed mares; however, they returned to below basal concentrations in feed-deprived mares, indicating a more efficient fatty acid utilization in these mares. Exercise-induced elevations in plasma NEFA concentrations for the use as metabolic fuel results from increased lipolysis via an insulin-related reduction in lipogenesis. During a 12 to 15 h period of feed

deprivation, elevated plasma NEFA concentrations were decreased during a warm up, increased during a walking phase, then decreased again during a high intensity phase of exercise (Lawrence et al., 1993).

Increased plasma β -hydroxybutyrate concentrations resulting from increased hepatic production (Heitmann et al., 1986) was reported in adult geldings and mares after 72 h of feed deprivation (Rose and Sampson, 1982). Similarly, in the present experiment, plasma β -hydroxybutyrate concentrations were elevated within 45 h and remained elevated through 69 h of feed withdrawal. Similar results were also observed in steers (Blum et al., 1981; Rule et al., 1985), adult sheep (Heitmann et al., 1986), and man (Ho et al., 1988; Beer et al., 1989).

In the horse, plasma lactate concentrations were reported to continually increase during 9 or 4 d without feed (Baetz and Pearson, 1972; Rose and Sampson, 1982). Likewise in these mares, plasma lactate concentrations were elevated during 3 d of feed deprivation. Increases in circulating lactate concentrations during feed deprivation would likely result from increased lactate production via muscle glycolysis for support of hepatic gluconeogenesis.

Elevated circulating lactate concentrations associated with exercise are likely the result of increased anaerobic metabolism via muscle or liver glycogenolysis, or glycolysis, and(or) decreased removal (Astrand and Rodahl, 1986). Deldar et al. (1982) and Rose and Sampson (1982) reported elevated plasma lactate concentrations after exercise in horses. This elevated lactate response was not influenced by a 12 to 15 h period of feed deprivation or ingestion of corn 3 h

before exercise (Lawrence et al., 1993). The responses of plasma lactate in this experiment (no influences of feed intake) coincide with those results reported by Lawrence et al. (1993).

Corresponding to results reported in these mares, Baetz and Pearson (1972) and Freestone et al. (1991) observed no changes in plasma glucose concentrations during 9 or 5 d of feed deprivation in adult ponies. Likewise, in swine, little variation of plasma glucose concentrations were observed during 48 h of feed deprivation (Baetz and Mengeling, 1971). Absolute requirements of glucose for the nervous system, erythrocytes and brain dictate a precise regulatory mechanism for the conservation of blood glucose (Murry et al., 1993). In man (Ho et al., 1988; Beer et al., 1989) and ruminants (Blum et al., 1981; Rule et al., 1985; Heitmann et al., 1986), plasma glucose concentrations have been shown to decrease during periods without feed intake.

Variations in the plasma glucose response to exercise has been observed in the horse. Corresponding to the present experiment, Lawrence et al. (1993) reported a reduction in circulating glucose concentrations after exercise in horses which consumed various amounts of corn 2.5 to 3 h before the exercise bout, but remained relative stable when they were held without feed for 12 to 15 h. The mares in this experiment also consumed their morning meal 3 h before initiation of exercise. Others have reported no fluctuations in plasma glucose concentration after exercise (Snow and Rose, 1981; Deldar et al., 1982; Rose and Sampson, 1982) or increased plasma glucose concentrations during endurance exercise (Snow and

Mackenzie, 1977). These inconsistent responses are likely related to either time of sampling relative to initiation of the exercise or to the previous feeding. Elevation of plasma glucose concentrations in the horse was observed through 8 h after feeding (DePew et al., 1994). Similar reduction in plasma glucose concentrations after exercise in mares that had eaten 3 h earlier might have also occurred without exercise. It is also likely that increased circulating glucose concentrations after a meal are used as an immediate energy fuel during exercise.

Rose and Sampson (1982) and Freestone et al. (1991) observed no modifications of plasma insulin concentrations during a 4 or 5 d period without feed in horses and ponies. Similarly, both plasma glucose and insulin concentrations were not influenced by feed deprivation in these mares. Insulin concentrations remained unchanged presumably because glucose is the major stimulator of insulin secretion; however, amino acids have also been implicated as insulin secretagogues (Davis, 1972; Chew et al., 1984b; Vicini et al., 1988). Reports in ruminants (Bassett, 1974b) and man (Ho et al., 1989) show decreased plasma insulin concentrations during feed deprivation.

Elevated plasma glucagon concentrations observed in these mares during feed deprivation are consistent with reports in humans (Beer et al., 1989). However, in ruminants, plasma glucagon concentration did not change during 3 or 8 d without feed (Rule et al., 1985; Heitmann et al., 1986). An elevation of plasma glucagon with feed deprivation would likely ensue from a reduction in postprandial glucose concentrations and(or) sympathetic stimulation. Bassett (1971) reported

elevated glucagon concentrations after epinephrine administration in sheep and Freeman (1980) concluded that glucagon is a stress hormone in chickens.

Baetz and Pearson (1972) reported reduced plasma urea N concentrations during 3 d, whereas Patterson et al. (1985) reported elevated plasma urea N concentrations during 4 d of feed deprivation in horses. Others reported increases in plasma urea N during 115 h of feed deprivation in swine (Baetz and Mengeling, 1971) and during 5 or 7 d in cattle (Blum et al, 1981; Blum and Kunz, 1981). Rule et al. (1985) observed an elevation after 2 d then a reduction in plasma urea N during the remaining 6 d of feed deprivation in cattle. Elevated plasma urea N concentrations were also observed in these mares during 21 to 69 h of feed withdrawal presumably related to increased protein mobilization for an energy fuel.

Other researchers have observed reductions in plasma IGF-1 concentrations during feed deprivation comparable to those observed in the present study. Buonomo and Baile (1991) reported a decrease in plasma IGF-1 concentrations in swine 48 h after feed removal and increased plasma IGF-1 with refeeding. Likewise, plasma IGF-1 concentrations decreased with feed deprivation in rats (Phillips and Young, 1976), humans (Merimee et al., 1982), and bulls and steers (Ward et al., 1992).

An elevation of plasma cortisol concentrations during feed deprivation would presumably be related to stress may function in nutrient mobilization; however, varying responses are observed. In adult ponies, plasma cortisol concentrations did not vary during 5 d (Freestone et al., 1991) or during 4 d without feed (Rose and

Sampson, 1982). Similarly, in the present experiment, feed deprivation did not influence plasma cortisol concentrations. On the other hand, in humans, 72 h of feed deprivation increased plasma cortisol and ACTH concentrations (Beer et al., 1989). Exercise is associated with the activation of the hypothalamo-pituitary-adrenocortical system because of the related stress (Malinowski et al., 1993). As observed in the present experiment, exercise increases plasma cortisol concentrations during or following exercise in horses (Martinez et al., 1988; Colborn et al., 1991b; Malinowski et al., 1993). However, feed deprivation did not alter this response to exercise.

Reduction of thyroid hormones during feed deprivation has been observed in several species and is attributed to either a reduction in thyroid stimulating hormone or decreased thyroid hormone secretion via increased circulating cortiosteroids (Beer et al., 1989). Even though feed deprivation did not alter either plasma T_3 or T_4 concentrations in these mares, others reported reduced plasma T_3 and T_4 concentrations in feed deprived sheep (Blum et al., 1980), cattle (Blum et al., 1981; Blum and Kunz, 1981; Tveit and Larsen, 1983), and pigs (Buonomo and Baile, 1991).

Plasma prolactin concentrations were not different during feed deprivation in these mares. This was likely the result of reduced plasma prolactin concentrations during the winter months in horses as observed by Thompson et al. (1987). DePew et al. (1994) reported low plasma prolactin concentrations after 15 h without feed. Others have reported similar results in rats held without feed for

72 h (Takahashi, et al., 1986), heifer calves for 60 h (McAttee and Trenkle, 1971c), and goats for 8 h (Bryant et al., 1970).

Elevated plasma GH during feed deprivation has been observed in 70 kg barrows (Buonomo and Baile, 1991), lambs or adult sheep (Bassett, 1974b; Cole et al., 1988), and adult humans (Ho et al., 1988; Beer et al., 1989). However, Rule et al. (1985) reported reduced plasma GH concentrations in steers, whereas McAttee and Trenkle (1971a) reported no change in plasma GH concentrations in calves during feed deprivation. In this experiment, 45 h of feed deprivation did not alter plasma GH secretion; however, 73 h of feed deprivation increased basal plasma GH concentrations which were taken before exercise. In addition, the GH response to exercise was greater in those mares which were held without feed compared with the mares which were fed. Exercise is a more potent stimulus for GH secretion than insulin hypoglycemia, arginine infusion, or L-DOPA administration, and as predictive as insulin hypoglycemia (Sutton and Lazarus, 1976). Thompson et al. (1992) also reported a greater GH response to exercise in stallions than either sexual stimulation, restraint via a twitch, or epinephrine administration. Therefore, from the results in this experiment, it appears that 73 h of feed deprivation increased pituitary GH stores and/or increased secretion of GH.

The effects of exercise on plasma GH and prolactin secretion, comparable to those reported for these mares, have been reported for horses (Thompson et al., 1992) and men (Luger et al., 1992). Saini et al. (1990) and Kramer et al. (1993) reported elevated plasma GH concentrations, but no responses of prolactin, during

exercise in men. Both Luger et al. (1992) and Kraemer et al. (1993) observed no variations in the response of GH and prolactin to exercise with state of training in men. Luger et al. (1992) reported that elevated plasma GH and prolactin concentrations in part were related to increased circulating lactate concentrations. Other mechanisms postulated to explain how exercise increases plasma GH and prolactin concentrations include brain neurotransmitters (Chiodini and Liuzzi, 1979) and sympathetic stimulation (Thompson et al., 1992).

Increases in concentrations of circulating GH (Thompson et al., 1992), epinephrine, norepinephrine (Snow and Rose, 1981), glucagon, cortisol, and lactate and decreases in concentrations of circulating insulin during exercise (Lucke and Hall, 1980) initiate nutrient mobilization through gluconeogenesis and lipolysis. The specific role of prolactin relative to exercise is unknown, but increases in plasma prolactin concentrations may aid in conservation of stored protein (Bauman et al., 1982).

In summary, increases in plasma NEFA, β -hydroxybutyrate, urea N, glucagon, cortisol, and GH, and decreases in IGF-1 concentrations were observed during feed deprivation; no changes of plasma lactate, glucose, insulin, prolactin, T_3 , and T_4 concentrations were observed. Differing responses of plasma NEFA, glucose, prolactin, and GH concentrations after exercise were observed relative to feed intake; however, no differences of plasma lactate and cortisol concentrations were observed after exercise during feed deprivation.

Implications

These results provide a comprehensive description of plasma metabolite and hormonal alterations during 73 h of feed deprivation and the resulting alterations during exercise. Responses of plasma β -hydroxybutyrate, glucagon, and IGF-1 concentrations to feed deprivation appear to follow previous reported results with other species. Plasma GH concentrations were not altered in mares which were feed deprived for 45 h; however, plasma GH concentrations were increased by 73 h of feed deprivation and peaked higher after exercise in the feed-deprived mares.

CHAPTER VII

OVERALL SUMMARY AND CONCLUSIONS

From the first experiment, it was concluded that restriction of protein and(or) energy altered plasma NEFA, urea N, and IGF-1 concentrations within 24 h of initiation of dietary treatments and did not influence plasma glucose, insulin, prolactin, and thyroid hormone concentrations. Protein restriction increased plasma GH episodes. Energy restriction altered the plasma NEFA response to feeding and epinephrine administration, whereas both protein and energy restriction altered the responses of plasma glucose and insulin concentrations to glucose administration. The second experiment confirmed the immediate responses of plasma NEFA concentrations to dietary alteration. Rapid alterations were also observed for plasma glucose and insulin during the 24-h feeding periods during which diets were switched. This experiment provided evidence of the importance of early sampling when monitoring plasma constituents during nutrient alterations and also showed that prefeeding responses of plasma metabolite and hormonal concentrations alone will not fully explain the metabolic consequences of nutrient restriction. The third experiment showed that plasma NEFA, β -hydroxybutyrate, urea N, glucagon, and cortisol were altered, whereas lactate, glucose, insulin, prolactin, thyroid hormones, and GH were unchanged during feed deprivation. Feed deprivation modified the responses of plasma NEFA, glucose, and prolactin to exercise, but did not influence the responses of lactate, cortisol, and GH. These results provide a comprehensive

reference for the responses of plasma metabolites and hormones to dietary protein and(or) energy restriction or feed deprivation.

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VITA

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Leeann was married on May 22, 1982 to Bradley A. Sticker. They have one son, Alan L. Sticker, born on November 18, 1987.

DOCTORAL EXAMINATION AND DISSERTATION REPORT

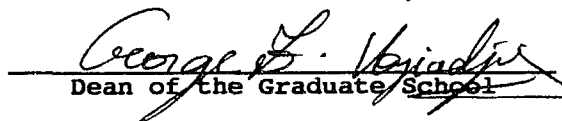
Candidate: Leeann Salathe Sticker

Major Field: Animal Science

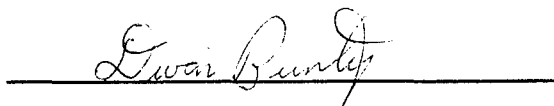
Title of Dissertation: Nutrient Restriction in Mares: Plasma Metabolite and Hormonal Characterization and Responses to Feeding and Metabolic Challenges

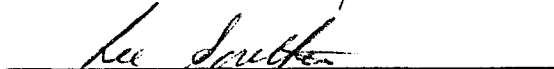
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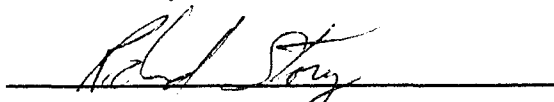

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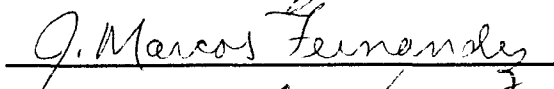

Dean of the Graduate School

EXAMINING COMMITTEE:











Date of Examination:

April 8, 1994